GEOMAR, Helmholtz Centre for Ocean Research, Kiel

Master Thesis

Seasonal condition and growth performance of Eastern Baltic cod larvae based on RNA/DNA ratios

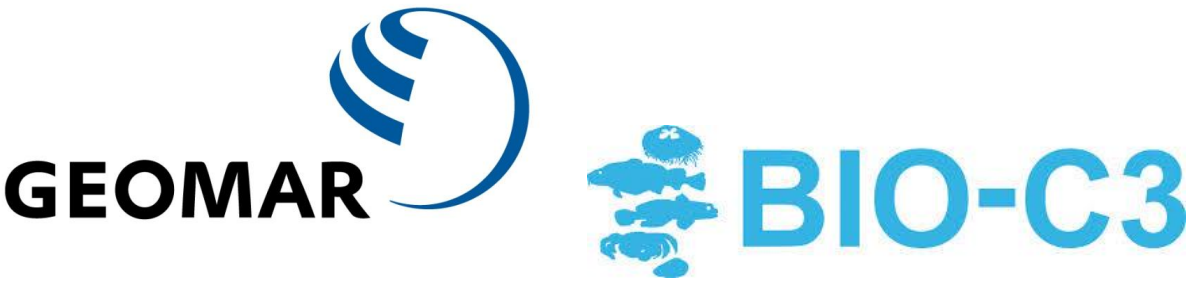
by Luisa Berghoff

Matr.No.: 996907

supervised by Dr. Catriona Clemmesen,

Dr. Bastian Huwer and Dr. Holger Haslob

examined by Prof. Dr. Thorsten Reusch and Dr. Catriona Clemmesen



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1 Summary

During the second half of the 20th century, anthropogenic exploitation and changes in atmospheric forcing resulted in high variations in recruitment and size of the Eastern Baltic cod stock. Successful spawning of Eastern Baltic cod is presently restricted to the most westerly deep basin of the Baltic Sea, the Bornholm Basin. Within the Bornholm Basin, high variability in environmental conditions has shown to be an important driver for the successful development of cod early life stages. The larval stage has been suggested to be important in shaping year-class strength as survival rates of fish larvae can be limited during different 'critical periods' depending on prevailing abiotic and biotic conditions. Annually performed ichthyoplankton surveys collect samples from the main spawning ground of Eastern Baltic cod to determine abundance and distribution patterns of cod early life stages and results for the year 2011 revealed highest larval cod abundance (ca. $70 \cdot 10^9$) since the mid-1980s (ca. $110 \cdot 10^9$). Abundances of Baltic cod larvae were unexpectedly low in 2004-2010 and high in 2011-2012. Larvae analysed in this study came from the high-abundance year 2011 and additionally provided data from 2006 and 2007. Hence, information on both, years of high and low abundance of cod larvae were available allowing for a direct comparison of contrasting situations in the field. The seasonal nutritional condition and growth performance (G_{pf}) of Eastern Baltic cod larvae in the years 2006, 2007 and 2011 were analysed based on the RNA and DNA ratio (R/D ratio) of whole larval tissue. The R/D ratio is a biochemical indicator for the recent nutritional condition and can be further converted into estimates of larval growth rate and growth performance. Furthermore, for 2006 and 2011 the R/D based nutritional condition of Baltic cod larvae was related to available data on the phenology, abundance and biomass of the main zooplankton species. This study demonstrated larval abundance, nutritional condition and growth performance to vary on an inter- and intra-annual basis. Both, nutritional condition and growth increased with larval size. Baltic cod larvae in the year 2011 presented higher nutritional condition and G_{pf} than in the years 2006 and 2007, mainly driven by high values in May 2011. Comparison with literature-derived reference growth rates revealed that Baltic cod larvae generally grow poorly. Latest sampling months at the end of the spawning season in November 2006 and 2011 presented relatively good nutritional condition of cod larvae. Good correlation between the nutritional condition of cod larvae and the occurrence of early developmental stages of the main copepod species, with

highest importance of *Pseudocalanus* spp., was found. Relatively high nutritional condition and growth rates in November, in combination with low abundances of the main copepod species, led to the assumption that high numbers of specimens of the rotifer genera might have a positive influence on cod larvae. This study provides evidence from the field that Baltic cod larvae might depend strongly on the spatial and temporal match and the magnitude of early developmental stages of the species *Pseudocalanus* spp. in order to increase their nutritional condition. Higher nutritional condition potentially increases the growth rate of Baltic cod larvae which can further result in reduced mortality. Thus, variability in larval nutritional condition and growth has a high potential to contribute to variability in year-class strength.

2 Introduction

2.1 Cod in the Baltic Sea

Since the mid-20th century, when the exploitation of the Baltic Sea became more intense, Atlantic cod (*Gadus morhua*) has been a popular hence commercially important fish species of the Baltic Sea (Sparholt 1994, Eero *et al.* 2008). Cod is a demersal species that preys on other fish (e.g. herring and sprat in the Baltic) as well as on various invertebrates (e.g. *Saduria entomon* and *Harmothoe sarsi* in the Baltic). Depending on the life-history stage of cod, habitats are variable and include shallow coastal waters down to greater depths of the continental shelf. The eggs are pelagic and also the larvae and the small juveniles stay in the pelagic zone until the juveniles settle to the bottom at a length of ca. 5 cm (Hüssy *et al.* 1997) and go over to a demersal life-style. In general, juveniles occur in nursery grounds (e.g. sea grass beds) being less than 10 to 30 m deep and providing shelter against predation (Cohen *et al.* 1990, Uzars and Plikshs 2000), while adults usually prefer deeper and colder water.

The species *Gadus morhua* is characterised by different subpopulations forming distinct spawning stocks geographically inhabiting the North Atlantic, the Arctic and the Baltic Sea. Within the Baltic Sea, cod is managed as two separate stocks, the Western and the Eastern Baltic cod stock (Bagge *et al.* 1994), which also show a number of biological differences and are genetically distinct (Hüssy *et al.* 2016). The management area of the Western Baltic cod is located in the Belt Sea, the Sound and the Arkona Basin (Subdivision (SD) 22 – 24; ICES 2012). The management area of the Eastern Baltic cod (further referred to as Baltic cod) stock is located in the Central and Eastern Baltic inclusive of the Bothnian Sea, Bothnian Bay and the Gulf of Finland (ICES SDs 25-32). In the past, Baltic cod spawning grounds were concentrated on the three major Baltic basins: the Bornholm Basin, the Gdansk Deep and the Gotland Basin (Fig. 1, ICES subdivisions 25-32, Bagge *et al.* 1994, MacKenzie *et al.* 1996).

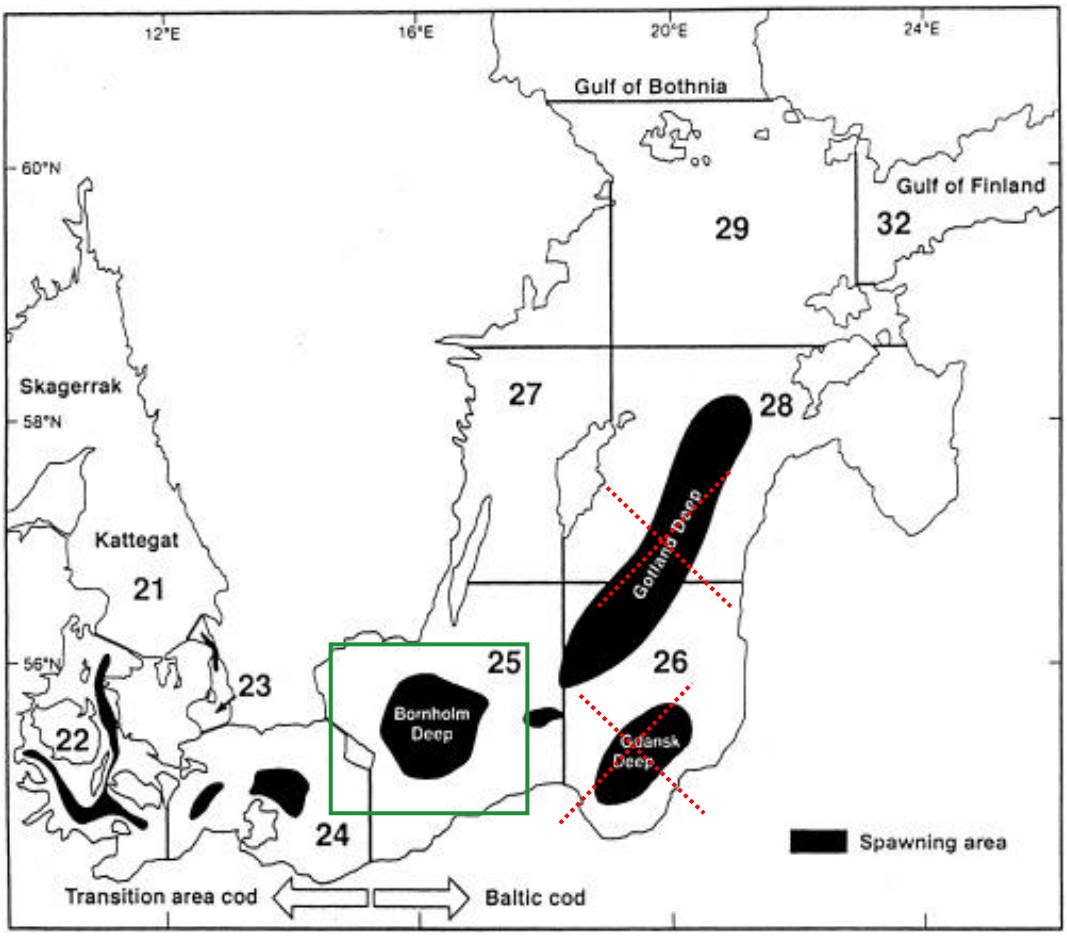

Fig. 1: Map of the Baltic Sea showing the location of cod spawning areas (black) and ICES subdivisions (SD, numbers) for the management of important fish species in the Baltic Sea. The management area of the Eastern Baltic cod stock extends from SD 25 to SD 32. The red dashed lines indicate the loss of the two spawning grounds for Eastern Baltic cod in the Gdansk Deep and the Gotland Deep since the late-1980s (Köster *et al.* 2005). The green line around SD 25 shows the recently only spawning ground of Eastern Baltic cod in the Bornholm Basin. The Fig. was modified after Bagge *et al.* (1994).

Within these deep basins a permanent halocline persists throughout the year (Elmgren *et al.* 1984) enabling cod eggs to float naturally at great depths. Due to changes in climatic forcing, the lack of inflows of oxygen-rich, highly saline water from the North Sea and the resulting increase of areas with persistently low oxygen, the Gdansk Deep and the Gotland Basin became more and more unsuitable environments for cod eggs. At present, the Bornholm Basin is therefore considered as the only spawning ground for Baltic cod where successful reproduction is possible (Fig. 1, ICES 2012).

2.2 Historical development of stock size, fishing mortality and recruitment

The International Council for the Exploration of the Sea (ICES) has been assessing the stock biomass of cod in the Baltic Sea since 1966 down to the present day. Since 2014, problems in the analytical stock assessment of Eastern Baltic cod have led to the recent use of spawning stock biomass (SSB) indices from BITS surveys (Baltic International Trawl Surveys) for estimation of a SSB proxy (ICES 2014).

With the initiation of assessment by ICES, the stock biomass of Baltic cod showed extreme fluctuations. Relatively low spawning stock biomass (SSB) was recorded during the 1960s (about 200.000 tonnes), followed by a heavy increase reaching historically high levels during the early 1980s (about 700.000 tonnes). From the early 1990s to 2006 cod biomass has been below average. By extending the time series back to 1946, Eero *et al.* (2007) showed Baltic cod to have been relatively heavily exploited during the second half of the last century. Exploitation in conjunction with disadvantageous environmental conditions, led to a fast decline in cod abundance during the mid-1980s. As fishery management has improved, fishing mortality between 2008 and 2011 has been estimated to be the lowest in the time series. Between 2006 and 2009 investigations by ICES (2012) calculated Baltic cod recruitment (at age 2) to be above average of the last 20 years. However, the combination of increasing recruitment and hence stock biomass and an apparently low amount of available food for cod has led to a reduced mean weight of older cod (4-7 years) since 2007 (Eero *et al.* 2012, 2015, ICES 2012, 2013).

2.3 Requirements for Recruitment

In order to define the key processes determining the fluctuations in SSB and recruitment success of Baltic cod, a variety of investigations and modelling on the early life history stages of Baltic cod have been conducted (e.g. Grønkjær and Wieland 1997, Wieland *et al.* 2000, Möllmann *et al.* 2000, Hinrichsen *et al.* 2002, 2005, Köster *et al.* 2003a, 2005, Huwer *et al.* 2011). Investigations on hydrographical prerequisites in the deep Basins, triggering important processes within the reproductive cascade of cod (gonad maturation, fertilization, egg survival, hatching rate, larval behavior), generated the concept of the so-called “Reproductive Volume” (RV; Westin and Nissling 1991, Nissling and Westin 1991, Nissling and Vallin 1996, Köster *et al.*

2005). The RV describes the volume of water, which provides at least the physical lower limits ($O_2 \geq 2 ml \cdot l^{-2}$; salinity ≥ 11, and temperature $\geq 1.5°C$) for successful reproduction of cod.

Considering the stratification of the Baltic Sea characterised by I) a permanent halocline at great depths separating water layers of different densities and II) a thermocline during summer months caused by surface heating, the proliferation of oxygenated saline water into the basins is dependent on horizontal water inflows from the North Sea. In the past, these so-called Major Baltic Inflows (MBI´s) were recorded every 1 to 3 years, while changes in climatic forcing reduced the frequency and magnitude of intervals to approximately every ten years since the early-1980s (Feistel *et al.* 2008). During stagnation periods oxygen contents below and within the halocline are reduced creating an unsuitable habitat for many organisms. The last major salt water inflows were reported in 1993 and 2003, a smaller one in 2006 and again in 2014 (Schinke and Matthäus 1998, Meier 2007, Nausch *et al.* 2013, Mohrholz *et al.* 2015). The frequency and intensity of these inflows has been suggested to have an influence on the recruitment success of Baltic cod. Köster *et al.* (2005) reported higher egg abundances after the MBI in 1993, but the abundances were not represented by higher larval abundances and recruitment levels in subsequent years. Earlier investigations by Köster *et al.* (2003a) already found that higher egg survival rates were not correlated with high abundance of larvae. However, larval abundances were correlated with recruitment level. Despite an increased SSB of Baltic cod, assessments on the reproductive volume by the ICES/HELCOM Working group on Integrated Assessments of the Baltic Sea (WGIAB; ICES 2013) indicated poor abiotic conditions for cod recruitment of the year-classes 2010 and 2011. However, the year-class of 2011 appeared to be above the recent average with highest estimations of recruits at age-2 (174 mill.) since 1988 (ICES 2014). Also abundances of Eastern Baltic cod larvae in 2011 were reported to be exceptionally high throughout the entire spawning season (Köster *et al.* unpubl., Holger Haslob and Bastian Huwer, pers. comm.), indicating the importance of processes influencing early life history stages and especially the survival of larvae for successful recruitment.

2.4 Sensitivity of Early Life History stages

Considering the different developmental stages of Baltic cod fish, high egg mortalities are mainly caused by above–mentioned unfavourable hydrographical conditions and to a certain extent by clupeid predation (Köster and Schnack 1994, Köster and Möllmann 2000, and Köster *et al.* 2005). Recent investigations by Neumann *et al.* (2014) found changes in the ichthyoplankton abundance and composition in the diet of the two Baltic clupeid species herring (*Clupea harengus*) and sprat (*Sprattus sprattus*), indicating reduced predation pressure in the latest period, potentially contributing to the improvement of cod recruitment.

Cod eggs in the Bornholm Basin usually occur in depths below 55m with a peak in abundance between 60 and 70 m (Bagge *et al.* 1994). After hatching, cod larvae need to conduct an ontogenetic vertical migration in order to prey on suitable copepod developmental stages in shallower water layers, followed by a reversed migration at increasing sizes into deeper layers providing cooler temperatures and larger prey items (Grønkjær and Wieland 1997, Grønkjær *et al.* 1997, and Huwer *et al.* 2011). Despite variations in egg mortality, the larval phase may be the bottleneck for successful recruitment of fish (Köster *et al.* 2003a). The Critical Period hypothesis (Hjort 1914, 1926) already linked the fate of year-class strength to variability in the performance of larvae finding suitable and sufficient prey items during first-feeding (critical stage: transition from endo- to exogenous nutrition). The Critical Period hypothesis was further supplemented by the Match-Mismatch hypothesis (Cushing 1974, 1975, 1990) declaring the importance of timing in terms of hatching larvae and the production of suitable zooplankton prey. Especially for temperate fish, the match of fish production and prey field would result in a narrow time window (few days or weeks) allowing for larval survival.

Besides variable predation pressure by clupeids (e.g. Köster and Schnack 1994, Köster and Möllmann 2000) and fluctuations in circulation patterns influencing dispersion and/or retention of larvae (Hinrichsen *et al.* 2001, 2003a), prey abundance and composition have been found to be crucial for larval cod survival (Munk 1997, Hinrichsen *et al.* 2003b, 2005, Köster *et al.* 2005). The decline of the copepod species *Pseudocalanus* spp. during the last two decades is considered to be a trigger for a shift from a non-limited to a food-limited state for larval cod and also a shift in spawning time from late spring to summer months (Wieland *et al.* 2000, Möllmann *et al.*

2000, Hinrichsen *et al.* 2003b). Biophysical modelling approaches have been conducted in order to identify the probability of survival of Baltic cod larvae (e.g. Hinrichsen *et al.* 2001, 2002, 2003b). However, information on larval Baltic cod growth and survival and the resulting implications for recruitment success from field studies are still scarce, and have so far largely been limited to the main spawning time (e.g. Grønkjær and Wieland 1997, Grønkjær *et al.* 1997, Huwer *et al.* 2011). Thus, it is of high interest to further investigate the nutritional condition, growth and survival chances of Baltic cod larvae in the field on a broader seasonal and inter-annual scale in order to contribute to a better understanding of the factors that influence recruitment success and the development of the future stock biomass.

2.5 Nutritional Condition and Growth Performance

In order to analyse the survival success of early developmental stages of fish, laboratory experiments and – to a smaller extent – field-studies revealed the cellular ratio of RNA and DNA (R/D ratio) to be a suitable biochemical indicator reflecting the recent nutritional condition and growth rate (G) of larvae (e.g. Clemmesen 1994, Grønkjær *et al.* 1997, Buckley *et al.* 1999, Huwer *et al.* 2011, Peck *et al.* 2015, Foley *et al.* 2016). Along the lines of the ”stage-duration hypothesis” (Houde 1987, Anderson 1988), the energetic input during the larval phase is concentrated on growth and storage of energy is negligible (Folkvord 2005). As larvae increase in size, mortality due to starvation and predation are generally thought to decrease and hence growth and nutritional condition become important factors affecting the year-class strength (Caldarone *et al.* 2003, Buckley *et al.* 2006, Huwer *et al.* 2011).

The ribosomal RNA contributes to the biosynthesis of proteins and increases in concentration during growth phases depending on the prevailing nutritional condition of a larva. The DNA content of a cell remains about constant in either well-fed or starving larval fish. Variable nutritional conditions of larvae have been found to be reflected by the R/D ratio within a few days allowing for estimations of environmental conditions experienced by larvae shortly before the time of sampling (e.g. Clemmesen 1994, Caldarone *et al.* 2005, Peck *et al.* 2015, Foley *et al.* 2016). Further, R/D ratios have been found suitable to be converted into estimations of growth by usage of R/D-models. As fishes are poikilotherms, biosynthesis of proteins per unit RNA is

dependent on the surrounding temperature. Laboratory experiments revealed a positive linear relationship between R/D ratio, temperature and size-specific growth (e.g. Buckley *et al.* 1984, Folkvord *et al.* 1996, Otterlie *et al.* 1999). R/D-based growth models including temperature terms have been developed for cod only and for a cod-haddock data set (RD-T-models, Caldarone *et al.* 2005, Folkvord 2005, Buckley *et al.* 2008). Next to information on growth rates, the more objective, temperature and size independent growth performance (G_{pf}) can be estimated. By help of reference growth rates from laboratory reared, fast-growing larvae, field-observed specific growth rates can be standardized (size-temperature-dependent growth model, STDG model, Folkvord 2005). The quotient of maximum possible growth realized by a larva at given size and temperature is described by comparing field-observed growth rates with model-based predicted growth rates.

Nutritional condition and growth performance have been found to be suitable indicators to understand and detect key regulatory mechanisms and their variability determining survival and hence the inter-annual recruitment success (e.g. Clemmesen *et al.* 1997, Grønkjær *et al.* 1997, Folkvord 2005, Buckley *et al.* 2008, Huwer *et al.* 2011). The shift from a non-limited to a food-limited environment for Baltic cod larvae proposed by different studies (Möllmann *et al.* 2000, Hinrichsen *et al.* 2003b) suggests intra- and inter-annual variances in the nutritional condition and growth performance of larvae mainly depending on the encounter with suitable and sufficient abundances of prey organisms. Field samples investigated in this study originate from the seasons 2006, 2007 (only August) and 2011. Especially the year 2011 was of high interest as larval abundances were exceptionally high throughout this season, despite indications of poor abiotic conditions for successful reproduction of Baltic cod by the WGIAB (ICES 2013). Based on the analyses of nutritional condition, growth and growth performance of Baltic cod larvae, this study aimed to investigate inter- and intra-annual variation between these years, with additional consideration on the occurrence of suitable prey fields.

In summary, this study aimed to disprove the following null hypotheses:

$H_0$1: Nutritional condition and growth of Baltic cod larvae is consistent between the years.

$H_0$2: Nutritional condition and growth of Baltic cod larvae is consistent within the spawning season.

$H_0$3: The nutritional condition of Baltic cod larvae has no relation to the seasonal occurrence, abundance and stage composition of dominant zooplankton species.

3 Material and Methods

3.1 Field sampling

Cod larvae were caught in the Bornholm Basin of the Baltic Sea in 2006, 2007 and 2011. In this southern area of the Baltic Sea several ichthyoplankton surveys have been conducted throughout the years (Table 1). Cod larvae from these surveys were kindly provided by Bastian Huwer (DTU Aqua, Kopenhagen) and Holger Haslob (GEOMAR).

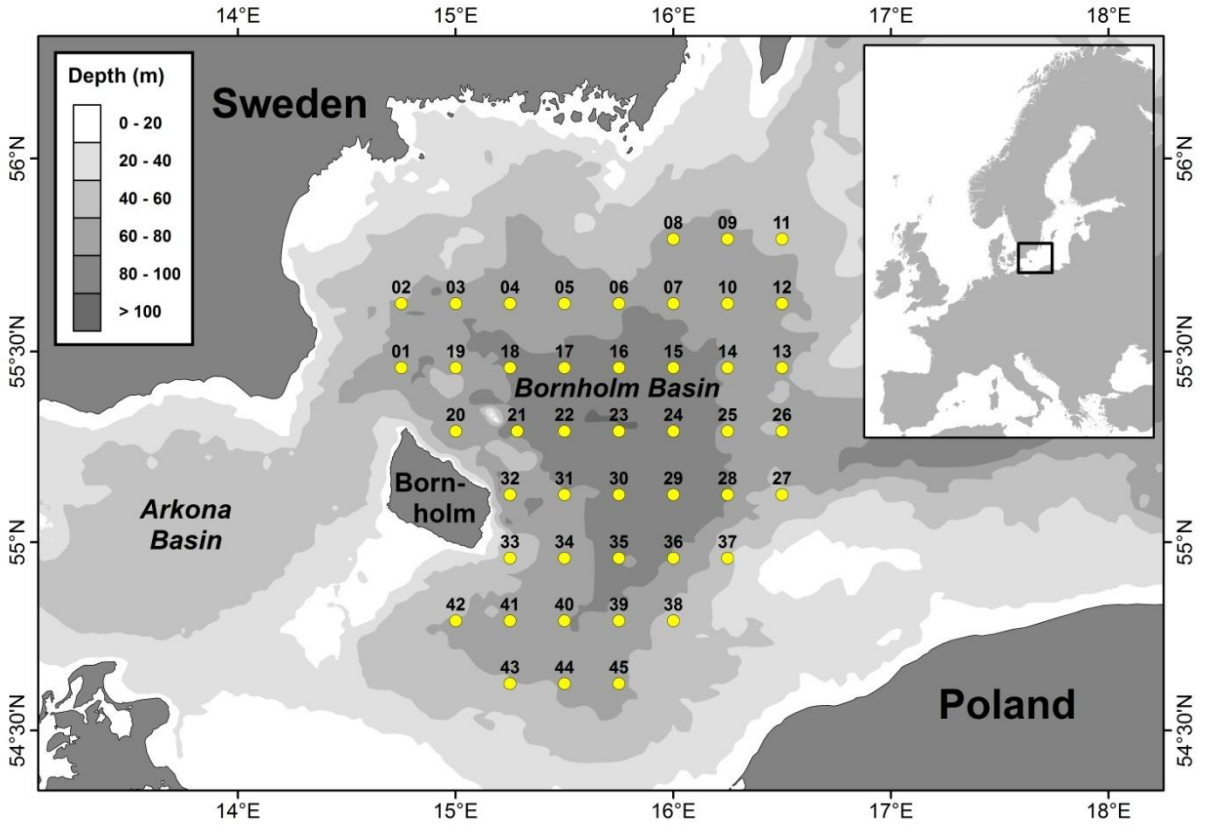

Fig. 2: Standard station grid for sampling of ichthyoplankton samples within the Eastern Baltic cod spawning area of the Bornholm Basin. Fig. provided by Bastian Huwer.

Within a standard station grid covering the area of the Bornholm Basin, double oblique hauls with a Bongo net were conducted (Fig. 2). During some cruises, additional Multinet (MN) hauls were deployed providing vertically resolved samples of 5m depth intervals (Table 1). For each station a hydrographical profile (temperature, salinity and oxygen concentration) was recorded

via CTD measurements. These CTD data were calibrated and allocated by Hans-Harald Hinrichsen (GEOMAR, Kiel). Once on board, cod larvae were quickly sorted out of the catch, pictures with an included scale bar were taken for later length analysis and each larva was put into an individual vial with seawater to be frozen at -80°C. Handling was conducted as fast as possible and samples were kept on ice preventing nucleic acids of larval tissue to degrade. Back at the respective institute, samples were stored again at -80°C.

3.2 Laboratory analyses

The ratio of RNA and DNA (R/D ratio) was determined according to the method described in Huwer *et al.* 2011. R/D data from 2006 and 2007 were kindly provided by Bastian Huwer. Cod larvae from the spawning season 2011 were analysed in the course of the year 2015 within this master thesis.

Pictures taken of each individual cod larva during the cruises were analysed by help of the software program image tool, determining the standard length (SL, mm) and taking notes on the larval appearance (e.g. number of eyes, shape). After unfreezing, cod larvae were gently washed in deionized water and after 30 minutes of refreezing at -80°C transferred into a freeze drier (Christ alpha 1-4) for at least 18 hours at -50°C and 0,100 mbar. The determination of larval dry weight (DW) in mg was conducted with a Sartorius SC2 micro balance. In order to facilitate later homogenization of larval tissue, differently sized glass beads were added into each vial (Sartorius, diameter: 2mm and 0, 17-0,5mm) to aid in mechanical disruption of the tissue. Starting from this step, samples were kept on ice. In consideration of the given DW, a defined volume of Tris-SDS buffer (Tris 0.05; NaCl 0,1M; SDS 0, 01%; EDTA 0, 01; pH 8) was added to the larvae and incubated for 30 minutes. After hydration of larval tissue, vials were put into a shaker (RETSCH type MM2, at intensity 100, and room temperature) for 15 minutes. and then transferred into a Sigma 3-18 K centrifuge running for 8 minutes at a speed of 6803 RPM (RCF: 3829g, temperature: 1°C). After centrifugation, the supernatant of each sample was either transferred into a new vial for further dilution steps or directly into a black 96-well-cliniplate.

Table 1: Overview of institutes and research vessels which provided larval cod samples from 2006 and 2011. Additional information on gear used during cruises: Bongo (60 cm mouth diameter, mesh size 500 µm), Multinet (MN, Hydro-Bios, Kiel, Germany, Type MAXI, aperture 71x71cm = 0,5m^2 and Type MIDI aperture 50x50cm = 0,25m^2, both mesh size 335µm).

Vessel	Cruise No.	Year	Month	Date	Gear	n stations	n cod larvae for analysis	Institute
ALKOR	AL 277	2006	April	19/04 to 05/05/06	Bongo	14	15	GEOMAR, Germany
ALKOR	AL 279	2006	June	31/05 to 17/06/06	Bongo	26	57	GEOMAR, Germany
ALKOR	AL 282	2006	July	17/07 to 31/07/06	Bongo/MN maxi	21	94	GEOMAR, Germany
ALKOR	AL 291	2006	November	06/11 to 14/11/06	Bongo	10	21	GEOMAR, Germany
ALKOR	AL 303	2007	August	09/08/ to 10/08/07	MN maxi	1	142	GEOMAR, Germany
DANA	DANA 02/11	2011	March	02/03 to 15/03/11	Bongo	7	11	DTU Aqua, Denmark
ALKOR	AL 373	2011	May	12/05 to 25/05/11	Bongo/MN maxi	15	106	GEOMAR, Germany
BALTICA	BA0611	2011	June	25/06 to 26/06/11	MN midi	1	128	MIR, Poland
ALKOR	AL 379	2011	August	11/08 to 26/08/11	Bongo/MN maxi	44	656	GEOMAR, Germany
DANA	DANA 08/11	2011	November	02/11 to 15/11/11	Bongo	18	43	DTU Aqua, Denmark

Larger larvae had to be diluted in order for their nucleic acid content to stay in the range of the defined calibration curves of RNA ($y=41.20(\pm4.24)\cdot x$; $R^2=0.998\pm0.001$; 16S-23S-ribosomal, Roche 10 206 938 001) and DNA ($y=80.47(\pm7.01)\cdot x$, $R^2 = 0{,}995 \pm 0.002$; λ-DNA, Roche 10 745 782 001) avoiding a loss in quality. For better accuracy, the calibration curve and a control homogenate consisting of larval herring tissue with a known R/D ratio were added to the 96-well-cliniplate on each day of laboratory analyses.

Before running the analyses, two integrated dispensers of the Ascent Fluoroscan (Thermo Fisher) were prepared with Ethidium bromide (EB, $2.5 mg\cdot ml^{-1}$ dilution, Roth 2218.2) and TE buffer (Tris 0.05; NaCl 0,1M; EDTA 0, 01; pH 8), respectively. Measurements were conducted at an excitation wavelength of 355 nm and an emission wavelength of 590 nm at a temperature of 25°C. For determination of the R/D ratio, fluorescence was measured in three steps: I) the pure samples (autofluorescence), II) after addition of EB (total nucleic acids) and III) the remaining DNA after incubation with RNAse (Serva Ribonuclease A, from bovine pancreas) for 30 minutes at 37°C. Subtracting the autofluorescence and the fluorescence of DNA from the total nucleic acid fluorescence led to the remaining fluorescence of RNA. By help of the calibration curves, relative fluorescence values could then be converted into µg values of RNA and DNA.

3.3 Determination of growth and growth performance estimates

In this study, the multi-species-model developed by Buckley *et al.* (2008) was applied (covering a temperature range of 3.4-28°C) to estimate weight specific growth (G). The model was based on a meta-analysis including 13 studies with information on R/D values of fish larvae, rearing temperature and their instantaneous growth rates.

Data of R/D ratios from the different studies were standardized based on the method developed by Caldarone *et al.* (2006). This method uses a common slope ratio of 2.4 in order to convert R/D ratios from different laboratories into standardized R/D values (sRD), delivering data comparable between different methods and laboratories.

Accordingly, R/D ratios of analyzed cod larvae from 2006 and 2011 were first standardized to a slope ratio of 2.4 and then used in the equation originating from the multi-species growth-model data set by Buckley *et al.* (2008):

$$G = 0.0145 * sRD + 0.0044 * sRD * T - 0.078 \quad (1)$$

Where G is the instantaneous growth rate, sRD is the standardized RNA/DNA ratio and T is the temperature experienced by the cod larvae.

For the estimation of larval growth rates, information on temperature experienced by each individual larva is essential as metabolic rates increase with higher temperature. Temperatures in the Bornholm Basin vary within the water column due to stratified water masses, in particular during summer when solar radiation results in a thermocline in the upper part of the water column (Fig. 3). Besides, larvae have been found to conduct an ontogenetic vertical migration (Grønkjær and Wieland 1997, Grønkjær *et al.* 1997, Huwer *et al.* 2011). Therefore, the accuracy of information on temperature experienced by the larva depends on the gear used for sampling. Sampling of larvae during the years 2006 and 2011 was conducted with either a Bongo net or a Multinet. Multinet samples enable vertically resolved sampling providing information at 5m depth intervals. As the conduction of Multinet hauls is rather time consuming, sampling of cod larvae during ichthyoplankton surveys is mainly conducted with Bongo nets. Bongo net hauls are not vertically resolved but rather integrate over the entire water column. Hence information on depth-dependent temperature at the exact sampling location was not available.

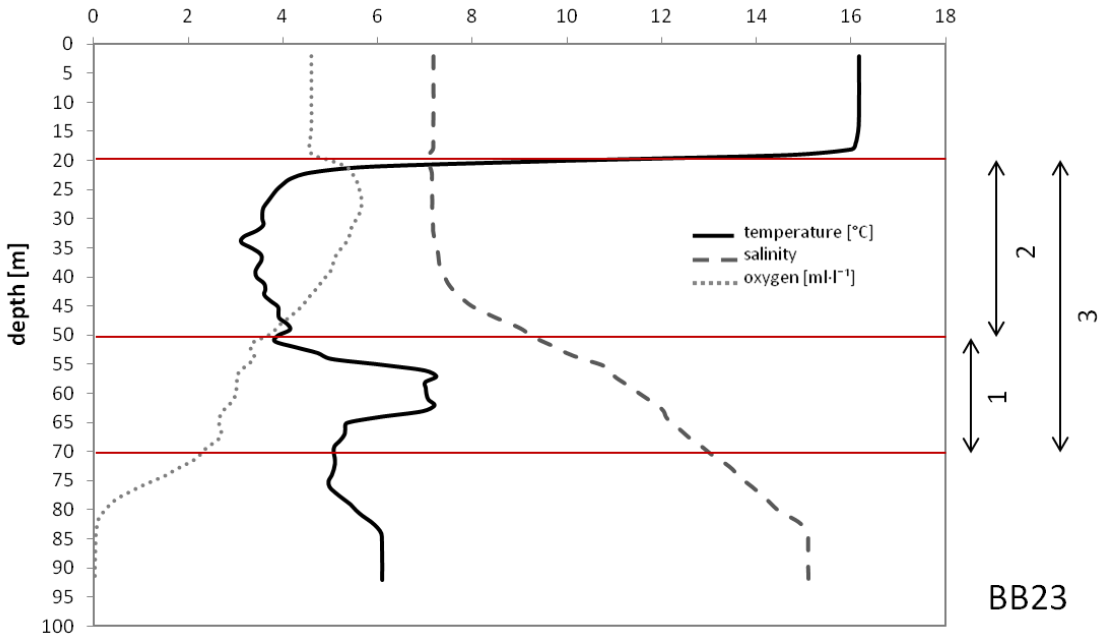

Fig. 3: Exemplary hydrographical profile at station BB23 in August 2011 with assumed size-dependent distribution patterns of Baltic cod larvae. Arrows and numbers 1-3 next to the graph indicate the assumed distribution patterns of Baltic cod larvae based on their standard length.

Field-based investigations on growth of Baltic cod larvae have focused on larvae sampled with a Multinet, providing information on the vertical distribution of cod larvae (e.g. Huwer *et al.* 2011). In order to gain information on growth rates of cod larvae originating from both Bongo net and Multinet hauls, a uniform approach for the calculation of temperature was developed based on information on general size-dependent vertical migration patterns of Baltic cod larvae found in the literature (Table 2). Literature-based migration patterns were further extended by information on size-dependent depth distribution of cod larvae obtained by Multinet hauls. Based on information on ontogenetically driven vertical migration, pre-feeding larvae (<4.5 mm) are assumed to be concentrated within hatching depths, reflected by the halocline (50-70 m, Fig. 3). In order to find suitable prey for initiation of first-feeding, larvae of 4.5 to 6 mm are considered to migrate from the halocline into shallower depths, while water temperatures above the thermocline usually appear to be high potentially causing thermal stress for cod larvae (20-50 m, Fig. 3). Finally, established feeding larvae (>6 mm) have been found to start a reversed migration into deeper waters due to increased larval size and the ability to feed on bigger prey located

within and below the halocline (20-50 m, Fig. 3; Grønkjær and Wieland 1997, Grønkjær *et al.* 1997, Huwer *et al.* 2011).

Table 2: Summary of size groups, their function and assumed vertical distribution modified after Grønkjær and Wieland 1997 and Huwer *et al.* 2011.

size class	SL [mm]	functional group	depth range [m]
1	< 4.5	pre-feeding yolk-sac larvae	50 - 70
2	4.5 - 6	first and early feeding larvae	20 - 50
3	> 6	established feeders	20 - 70

Based on this information, the methodology used for calculation of temperatures experienced by cod larvae was applicable for both sampling gears (Bongo and Multinet). Temperature means of the defined depth intervals were calculated at each respective sampling station and assigned to the different size classes (Table 2).

The growth rate G from equation 1 was then converted into specific growth rate (SGR, % d^{-1}) according to:

$$\mathrm{SGR} = 100 * (e^{G} - 1) \quad (2)$$

Field-based estimations of SGR can be compared to laboratory derived models producing suitable reference growth predictions. By help of reference growth rates (G_{ref}) of laboratory reared larvae, hypothetically growing at maximum rate, field-observed specific growth rates could be put into relation. In this study, G_{ref} was calculated according to the size-temperature dependent growth model (STDG-model) presented by Folkvord (2005) using the available data on dry weight and observed ambient temperature of sampled Baltic cod larvae. Observed biochemically derived growth rates were divided by growth rates predicted by the Folkvord model resulting in the so called growth performance (G_{pf}):

$$G_{pf} = \mathrm{SGR}/G_{ref} \quad (3)$$

This calculation allowed estimating the fraction of maximum growth realized by a larva of a given size and under a defined temperature. Hence, it was possible to obtain an objective growth calculation independent of the parameters size and temperature. According to limit values of the STDG-model, larvae with a dry weight < 30 µg were excluded from the G_{pf} analysis. Also all larvae with a negative reference growth rate were disqualified in terms of unrealistic growth conditions.

3.4 Zooplankton sampling (abundance/biomass)

Reaching beyond the mere knowledge on nutritional condition and growth of Baltic cod larvae, also the phenology of zooplankton species and hence possible match/mismatch situations with cod larvae were analysed in the years 2006 and 2011. These data were kindly provided by Jörg Dutz (IOW, Warnemünde). No data were available for the year 2007.

Parallel to ichthyoplankton sampling, additional Baby-Bongo (150 µm, 0.12 m^2 mouth opening) hauls were conducted at representative stations within the Bornholm Basin station grid (Fig. 2) in order to sample zooplankton. The samples were analysed in terms of copepod composition and their developmental stages. Taxonomic identification of copepods was conducted in the laboratory with a compound microscope. Copepods were divided into an overall nauplii stage (N1-N6 combined), 5 copepodite stages (C1 up to C5) and adult stages which further were differentiated into sexes (male and female). After counting and identification, the abundance of each species and stage was extrapolated by help of the filtered volume of water derived from flow meter readings. Abundances were given as individuals · m^{-3}. By help of species-specific conversion factors according to the HELCOM standards, abundances of copepods and other taxa were converted into estimations of biomass in µg C · m^{-3}. Investigations on the stomach content of cod larvae by Voss *et al.* (2003) showed that throughout the season cod larvae in the size range of 4 to 6 mm predominantly feed on nauplii stages. Copepodite stages of the species *Acartia* spp. were completely avoided with an active selection for *Pseudocalanus* spp.. Accordingly, the main focus was attributed to link the abundance/biomass of early developmental stages of *Pseudocalanus* spp. with the nutritional condition (standardized R/D) of

Eastern Baltic cod larvae. Additional analyses with the overall spectrum of sampled copepods and other taxa (cladocera and rotifers) were also conducted. For corresponding months, sRD of cod larvae was compared with different combinations of copepod developmental stages: I) N1-N6 + C1, II) N1-N6 + C1-C2 and III) N1-N6 + C1-C3. These developmental groups were pooled for all copepod species and *Pseudocalanus* spp. alone. The choice of the stage-groups was based on the fact, that nauplii could not be quantitatively sampled with a 150 µm Baby-Bongo. However, the occurrence of copepodite stage 1 premises a previous existence of nauplii.

3.5 Data and statistical analysis

Larvae >15 mm were excluded from the analyses, as most Atlantic cod species undergo a metamorphosis from larvae to juvenile approximately starting at a SL of 12 mm (Kvenseth and Oiestad 1984; Pedersen *et al.* 1989). Larvae were always considered as I) one group and as II) three different size classes based on their functional size group (Table 2). The length-frequency distribution of cod larvae in each month was checked for bimodal length distribution by application of the Hartigan's *dip test* statistic for unimodality. For the variables of sRD, growth and growth performance a multi-factorial analysis of variance (ANOVA) was conducted including the factors year, month and size class and their respective interactions. The data were checked for normal distribution and homogeneity of variance both visually and by application of suitable tests (parametric tests: Shapiro test, Fligner test; non-parametric tests: Kruskal-Wallis, Levene-test). Data were either square root or log transformed if necessary to fit assumptions.

4 Results

Eastern Baltic cod larvae analysed in this study originated from the spawning seasons 2006, 2007 and 2011. Only larvae with a standard length < 15 mm were analysed. Laboratory analyses were conducted on 944 larvae from 2011. Provided data from the years 2006 and 2007 comprised information on sRD and growth of 187 and 142 cod larvae respectively. The data from 2006 and 2011 were seasonally resolved originating from several sampling months, while data from 2007 were only sampled during August. Hence information on cod larvae originating from the year 2007 were exclusively included into analyses of larval growth performance.

4.1 Length-frequency distribution of Baltic cod larvae

Monthly means (±sd) of larval cod standard length for all size classes and in relation to the three size classes from the years 2006 and 2011 are displayed in Table 3. The relative length frequency-distribution is given for each sampling month (Fig. 4) with a 0.5 mm resolution of larval standard length. The standard length (SL) of larvae caught in 2006 ranged from 2.51 to 12.62 mm with a mean SL of 5.95 (± 2.02) mm. In 2011, minimum SL of cod larvae was 2.97 mm extending to a maximum of 14.5 mm with a mean of 5.93 (± 1.41) mm. Between the years, the proportion of the defined size classes (sc1: <4.5mm, sc2: 4.5 – 6 mm; sc3: >6mm) in the order sc1, sc2 and sc3 contributing to the total number of cod larvae was 28%, 34% and 38% in 2006 and 11%, 49% and 40% in 2011.

The mean SL of cod larvae during the first sampling month of the respective years were 4.46 (± 0.51) mm in April 2006 and 4.73 (± 0.58) mm in March 2011. In April 2006, the proportion of larvae in the size classes 1, 2 and 3 was 73%, 20% and 7%. For the same size groups, March 2011 showed following proportions: 27%, 73% and 0%. Throughout the year 2006 the mean SL of Baltic cod larvae increased up to 7.42 (± 2.59) mm in November. At that time, the contribution of larvae to the different size classes showed a shift from smaller to bigger larvae (sc1: 19%, sc2: 19% and sc3: 62%). In 2011, mean standard length of cod larvae increased to 7.22 (± 2.52) mm in November with 0%, 42% and 58% for the size classes 1, 2 and 3. The year 2011 was characterized by decreasing rates of sc2 (May: 69%, June: 55%, August: 45% and November: 42%) and increasing proportion of sc3 (May: 12%, June: 21%, August: 48% and

November: 58%). In both years statistical tests showed no bimodal distribution of larval standard length over the year (all $p>0.05$).

Table 3: Mean (±sd) of Baltic cod larval standard length (mm) and sample number (N) in each sampling month in 2006 and 2011. Cod larvae are considered both independent of size classes and in relation to size class 1, 2 and 3 (<4.5, 4.5 – 6 and >6 mm respectively). NA = not available. (*Note*: The numbers (N) of presented Baltic cod larvae originate from samples collected for biochemical analyses.)

Year	month	Size class	Mean ± sd	N	Year	month	Size class	Mean ± sd	N
2006	all	all sizes	5.95 ± 2.02	187	2011	all	all sizes	5.92 ± 1.41	944
		1	4.07 ± 0.42	52			1	4.16 ± 0.29	102
		2	5.14 ± 0.45	64			2	5.21 ± 0.40	461
		3	8.07 ± 1.68	71			3	7.27 ± 1.19	381
	April	all sizes	4.46 ± 0.51	15		March	all sizes	4.73 ± 0.58	11
		1	4.26 ± 0.16	11			1	3.93 ± 0.42	3
		2	4.63 ± 0.01	3			2	5.04 ± 0.23	8
		3	NA	1			3	NA	0
	June	all sizes	6.16 ± 2.38	57		May	all sizes	5.17 ± 0.82	106
		1	3.90 ± 0.53	15			1	4.19 ± 0.26	20
		2	5.18 ± 0.48	21			2	5.17 ± 0.38	73
		3	8.77 ±1.85	21			3	6.73± 0.85	13
	July	all sizes	5.73 ± 1.54	94		June	all sizes	5.31 ± 1.33	128
		1	4.06 ± 0.41	22			1	4.13 ± 0.28	31
		2	5.14 ± 0.40	36			2	4.98 ± 0.34	70
		3	7.36 ± 1.08	36			3	7.57 ± 1.01	27
	November	all sizes	7.42 ± 2.59	21		August	all sizes	6.10 ± 1.29	656
		1	4.32 ± 0.19	4			1	4.19 ± 0.30	48
		2	5.29 ± 0.69	4			2	5.27 ± 0.41	292
		3	9.04 ± 1.88	13			3	7.17 ± 0.98	316
						November	all sizes	7.21 ± 2.52	43
							1	NA	0
							2	5.32 ± 0.28	18
							3	8.59 ± 2.53	25

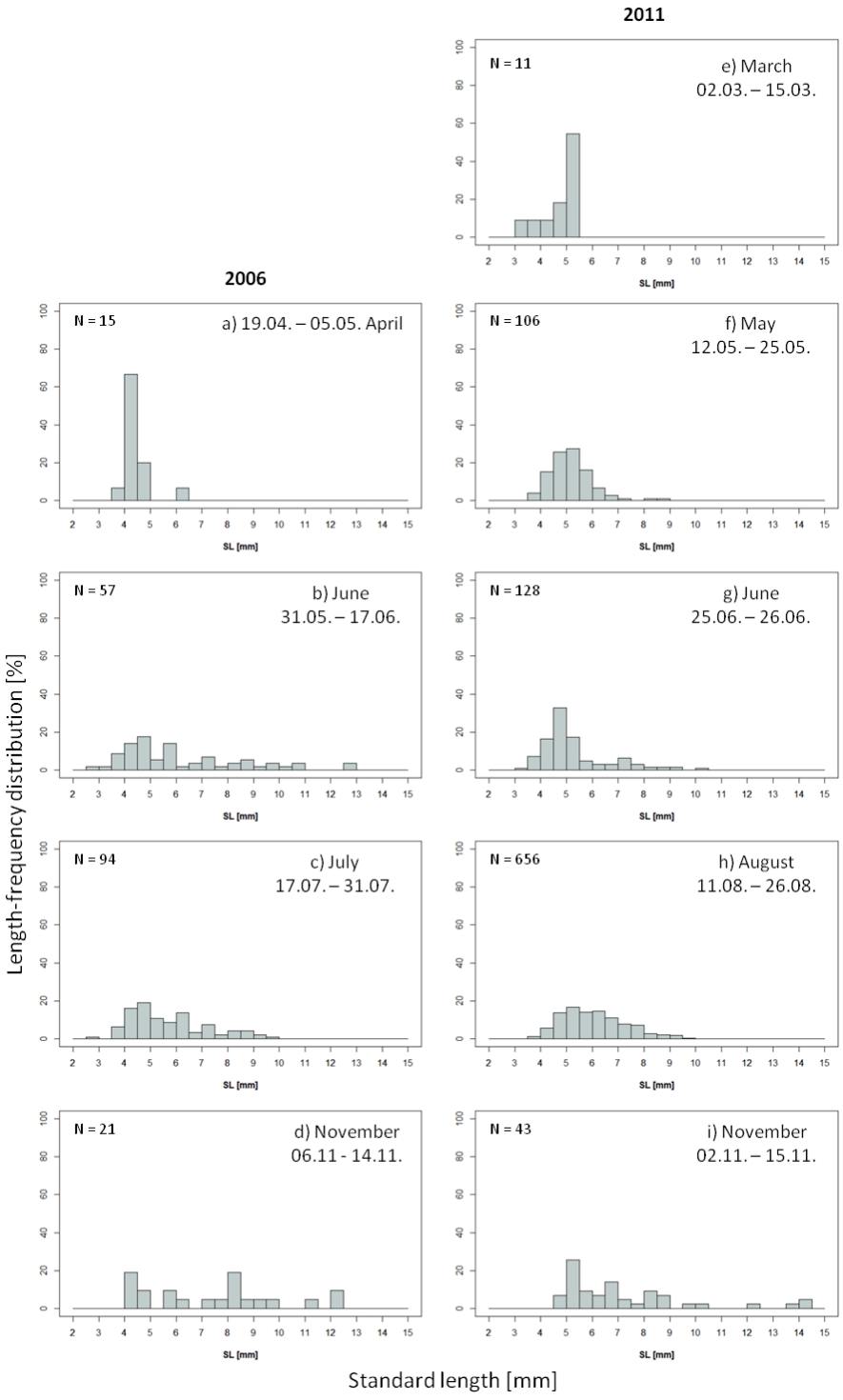

Fig. 4: Length-frequency distribution (%) of Baltic cod larvae caught in the Bornholm Basin over the sampling months in 2006 and 2011. (*Note*: The numbers (N) of presented Baltic cod larvae originate only from samples collected for biochemical analyses.)

4.2 Nutritional condition of Baltic cod larvae (standardized R/D ratios)

In the following, the results of standardized R/D ratios (sRD) in the years 2006 and 2011 are presented. Individual data on sRD ratios of Baltic cod larvae between the years of interest are shown in Fig. 5. Mean sRDs of all size classes and of the three functional size groups for different months over the two spawning seasons are graphically displayed in Fig. 6. Information on monthly means and standard deviations are given in Table 4.

The results of a multi-factorial analysis of variance showed significant influence of the factors year, month and size class on the sRD ratio of Baltic cod (Table 7). Interactions between the predictors were shown between year and month and month and size class ($p < 0.001$ respectively; Table 7).

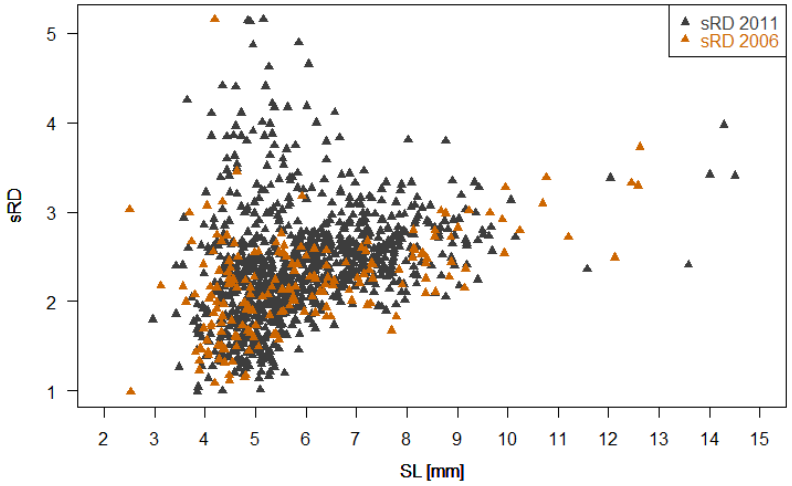

Fig. 5: Individual data on standardized R/D ratios (sRD) over standard length (SL, mm) of Baltic cod larvae in 2006 (orange) and 2011 (grey).

Between the years 2006 and 2011 differences in the overall mean sRD were found (2.24 (± 0.55) and 2.39 (± 0.62), respectively). With increasing standard length (SL) of Baltic cod larvae the mean sRD ratio also increased and variances decreased. Both years showed differences in mean sRD between sampling months (Table 4).

In 2006, overall highest mean sRD was found for June (Table 4, Fig. 6, left panel). The separation of cod larvae into three functional size groups presented a similar picture with each size class showing highest mean sRD during June. For larvae < 4.5 mm mean sRD was higher in the first two sampling months (April and June) than compared to the subsequent ones (Fig. 6c). Size class 2 (sc2, 4.5 – 6mm) displayed decreasing mean sRD from June to November (Fig. 6e), while values for larvae > 6mm (sc3) presented similar mean sRD between July and November (Fig. 6g).

For the year 2011, highest mean sRD for all larvae and also for each individual size class was found in May (Table 4, Fig. 6, right panel). After the peak of highest nutritional condition in May 2011, larvae of sc1 displayed similarly lower mean sRD in June and August (Fig. 6e). Larvae of sc2 and sc3 demonstrated overall lower mean sRD from June to November while the month August showed slightly higher values for sc2 and lower values for sc3 (Fig. 6f and h).

Table 4: Monthly means (±sd) of standardized R/D ratios of Eastern Baltic cod larvae in 2006 and 2011.

Year	**month**	**Size class**	**Mean ± sd**	**Year**	**month**	**Size class**	**Mean ± sd**
2006	April	all sizes	2.26 ± 0.59	2011	March	all sizes	1.49 ± 0.33
	June		2.57 ± 0.56		May		3.30 ±0.76
	July		2.05 ± 0.44		June		2.07 ± 0.58
	November		2.13 ± 0.53		August		2.30 ± 0.44
					November		2.42 ± 0.58
	April	<4.5 mm	2.16 ± 0.52		March	< 4.5 mm	1.63 ± 0.59
	June		2.54 ± 0.84		May		3.22 ± 0.67
	July		1.71 ± 0.48		June		1.89 ± 0.47
	November		1.63 ± 0.46		August		1.91 ± 0.48
	June	4.5 to 6 mm	2.35 ± 0.35		March	4.5 to 6 mm	1.44 ± 0.23
	July		1.98 ± 0.35		May		3.25 ± 0.82
	November		1.74 ± 0.38		June		1.86 ± 0.47
					August		2.14 ± 0.43
					November		1.96 ± 0.39
	June	> 6 mm	2.81 ± 0.41		May	> 6 mm	3.70 ± 0.45
	July		2.34 ± 0.30		June		2.83 ± 0.31
	November		2.41 ± 0.40		August		2.5 ± 0.33
					November		2.75 ± 0.45

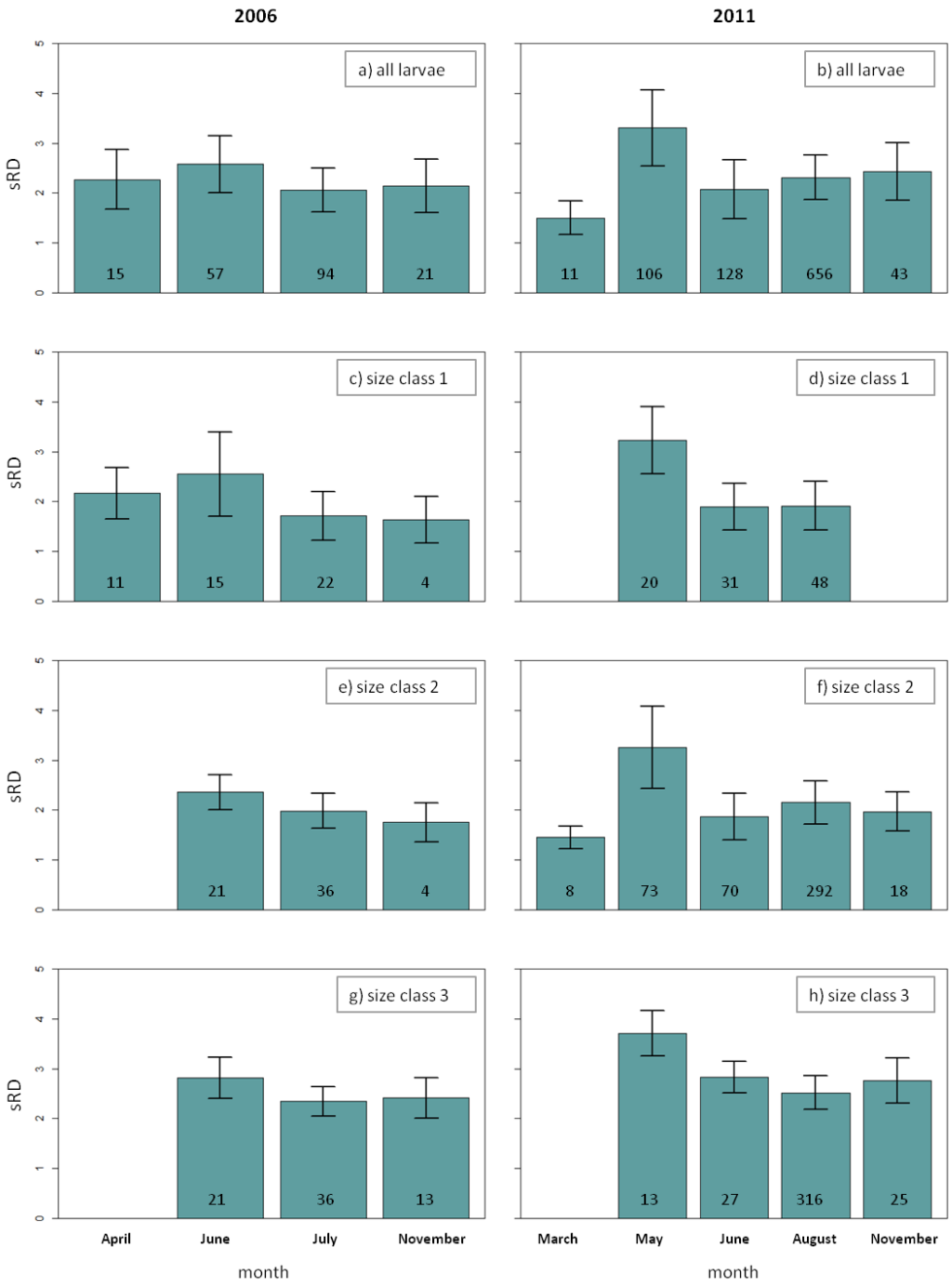

Fig. 6: Standardized R/D (sRD) ratios of Baltic cod larvae sampled in the Bornholm Basin in the course of the spawning seasons 2006 and 2011. Bar charts represent the mean (±sd) of all larvae independent of size class (a & b), size class 1 (c & d), size class 2 (e & f), and size class 3 (g & h). The sample sizes (N) for each year and month are presented in the respective bar charts. All N < 4 were excluded from the analyses.

4.3 Hydrographical profiles at representative stations in the years 2006 and 2011

The determination of size-dependent temperature values for the calculation of larval growth rates was based on CTD data at respective sampling stations. Representative stations within the Bornholm Basin are given for each month of the investigated years 2006 and 2011 in Fig. 7.

In both years, salinity increased in depths mainly around 50 m (Fig. 7 a-i) and a year-round halocline was observed. Water masses in the first sampling month in 2011 (March 2011, Fig. 7e) displayed evenly distributed temperatures within the water body above the halocline. With proceeding season, the development of a thermocline around a depth of 20 m could be observed (April 2006, Fig. 7a; May 2011, Fig. 7f). Hydrographical profiles in the latest sampling month November presented a reduction of the thermocline and a more homogenous distribution of temperature (Fig. 7d and i). The initiation of mixing water masses later in the season, caused overall higher temperatures in intermediate depths.

Between the months, variations in temperature means could be observed for all depth ranges (Fig. 7a-i). Overall, temperature values for sc1 averaged over all stations (respective mean of 50-70 m) were slightly higher 2006. Temperatures for sc2 (20-50 m) and sc3 (20-70 m) showed small variations from the earlier season until June in both years (Fig. 7a-b and e-g). Slightly increased temperatures in July 2006 almost doubled until the last sampling month in November (Fig. 7c and d). Warmer temperatures in August 2011 were only increased by around 1 °C in November (Fig. 7h-i).

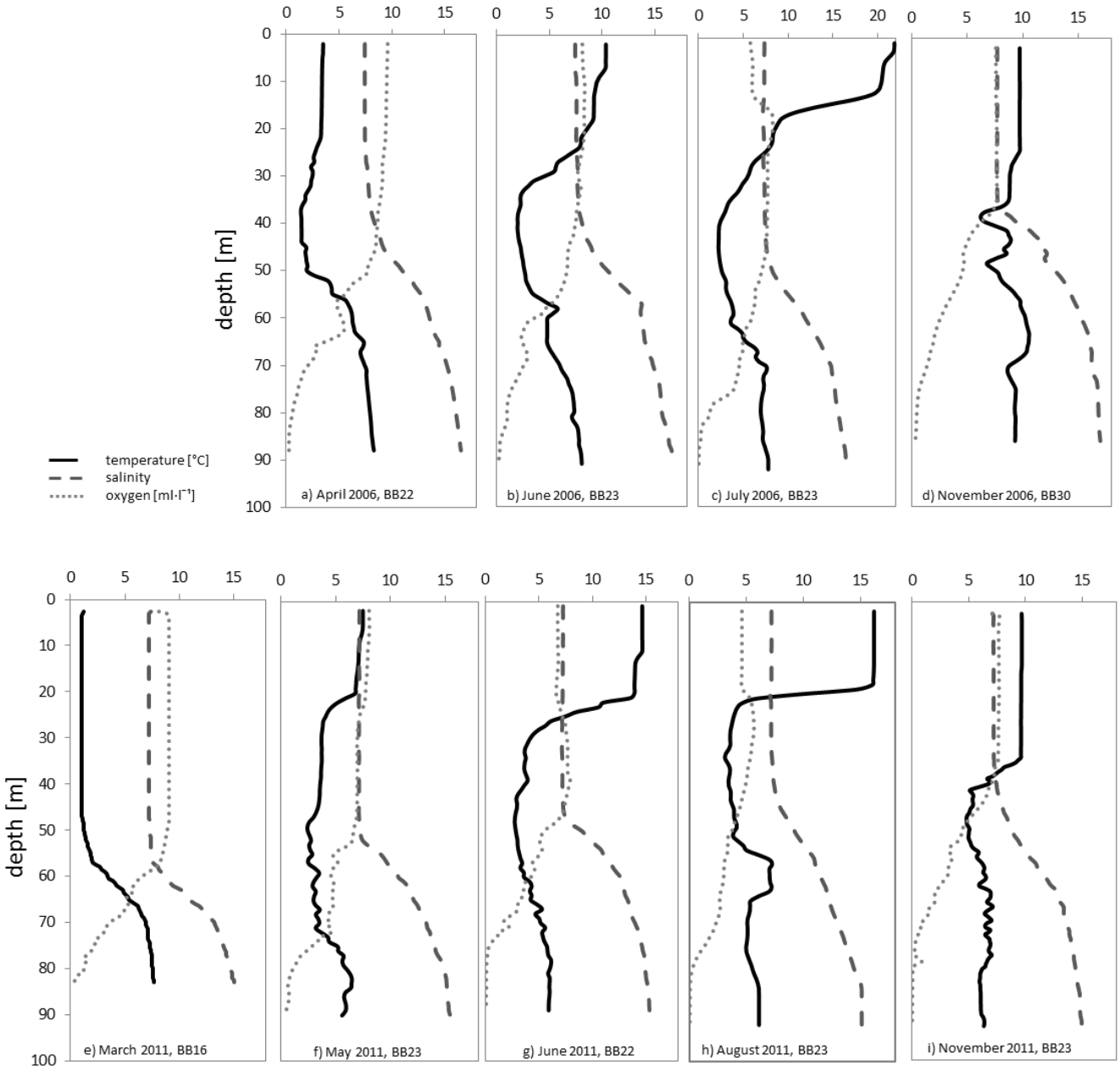

Fig. 7: Hydrographical profiles (temperature, salinity and oxygen) at representative sampling stations in the centre of the Bornholm Basin in the years 2006 (a-d) and 2011 (e-i).

4.4 Specific growth rate of Baltic cod larvae

Results on individual and mean specific growth rates (SGRs) of all larvae and also in relation to different size classes are shown in Fig. 8, 9 and 10. Specific growth rate calculations are based on the multi-species model by Buckley *et al.* (2008). The values of mean specific growth rates (± sd) are given in Table 5.

Between the years significant differences in specific growth rates were found (Fig. 8). With an average SGR of 1.69 (± 2.98) %·d^{-1} the year 2011 presented higher growth rates compared to 2006 (0.35 (± 2.37) %·d^{-1}). Results of a multi-factorial ANOVA showed significant influences of the factors year, month and size class on the specific growth rate of Baltic cod larvae (Table 7). Between the main factors, month and size class interacted most significantly ($p<0.001$), followed by the interaction of the factors year and month ($p=0.008$) and year and size class ($p=0.009$). No significant interaction between the three factors (year, month, size class) was found ($p=0.2$).

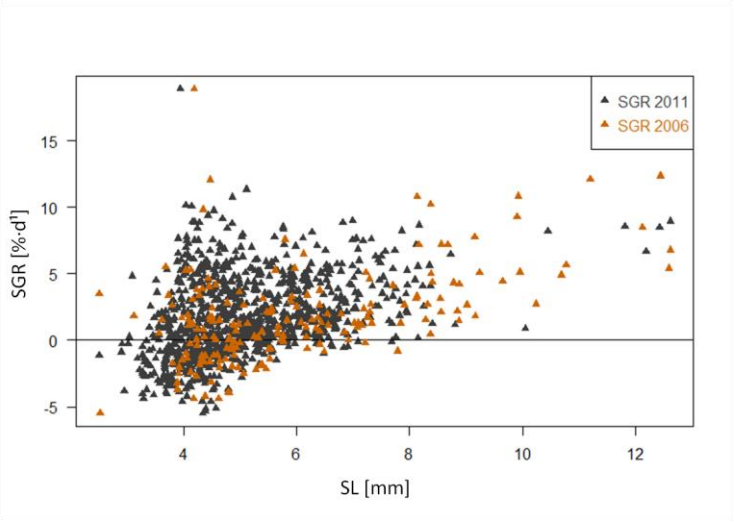

Fig. 8: Individual data on specific growth rates [% d^{-1}] of Baltic cod larvae in the years 2006 (orange) and 2011 (grey). SGRs were calculated according to the multi-species model developed by Buckley *et al.* (2008).

Overall, specific growth rates (SGR) in 2006 ranged from -0.45 (±1.75) to 3.49 (± 2.85) % increase in dry weight per day (Fig. 9a). For all larvae and also for the different size classes SGR was found to be highest in November (Fig. 9a-d). Larvae < 4.5 mm (sc1) displayed positive mean growth rates in April, June and November (Fig. 9b). Mean growth rates for size class 2

only presented positive growth during November (Fig. 9c), while larvae > 6mm (sc3) showed positive mean specific growth rates in all analysed months (Fig. 9d).

During the season 2011 the overall mean SGR of Baltic cod larvae varied over the months resulting in values ranging from -4.33 (± 1.30) to 3.51 (± 2.47) $\%\cdot d^{-1}$ (Fig. 10a). Considering all larvae independent of size, larval growth rates during November were found to be highest. For the different size classes, larvae of sc1 and sc2 showed highest mean SGR in May 2011 (Fig. 10b-c). Larvae belonging to sc1 presented positive mean growth rates only in May, while larvae of sc2 also presented positive growth in August and November. Considering larvae with a standard length > 6 mm (sc3), mean growth rates over the season 2011 were overall positive with highest mean SGR in November followed by the month May (Fig. 10d).

In order to demonstrate the influence on the outcome of specific growth rates of Baltic cod larvae, depending on the assigned temperature value put into the multi-species model, mean values of SGR for larvae sampled only with Multinets are shown in Fig. 10e-g. Vertically resolved samples allowed for calculations of temperature means over 5m depth intervals and a direct association to larvae sampled at respective depths. Fig. 10e shows mean SGRs of Baltic cod larvae < 4.5 mm ranging from -1.51 (± 1.94) to 4.57 (± 2.97) $\%\cdot d^{-1}$. While May presented highest average SGR, also mean growth rates in August were positive (4.13 (± 2.77) $\%\cdot d^{-1}$). Except for mean SGRs in June and August being slightly smaller, mean specific growth rates of size class 2 and 3 in May increased when the more precise temperature means from the MN hauls were used (Fig. 10f and g). SGRs in May were highest with an average value of 5.65 (± 3.91) and 11.67 (± 4.74) $\%\cdot d^{-1}$, respectively. Specific growth rates in June were on average -1.93 (±1.57) for sc2 and 0.33 (±1.06) $\%\cdot d^{-1}$ for sc3.

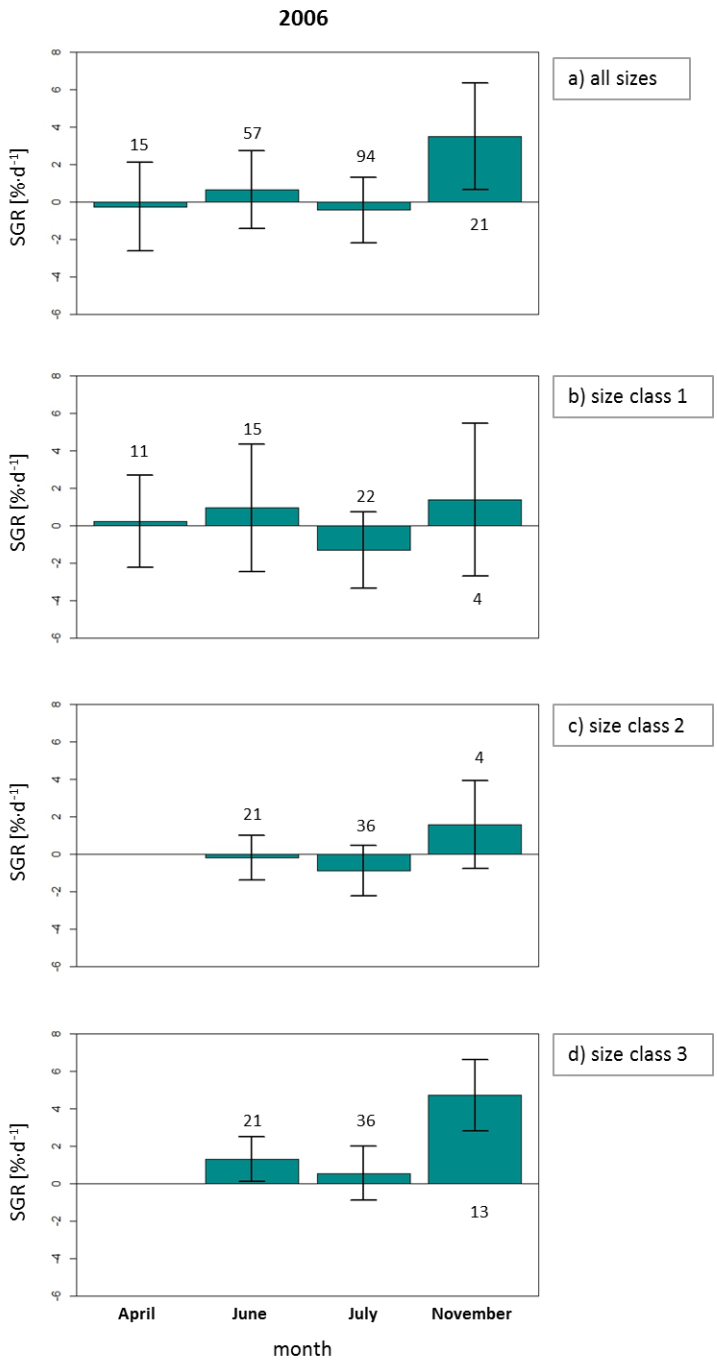

Fig. 9: Mean specific growth rates (SGR, % $\cdot d^{-1}$, ±sd) of Baltic cod larvae caught in 2006. SGR is given over the season and sample sizes (N) are shown above or below the respective bar chart. Bar charts represent the mean SGR of all size classes (a), size class 1 (b), size class 2 (c) and size class 3 (d).

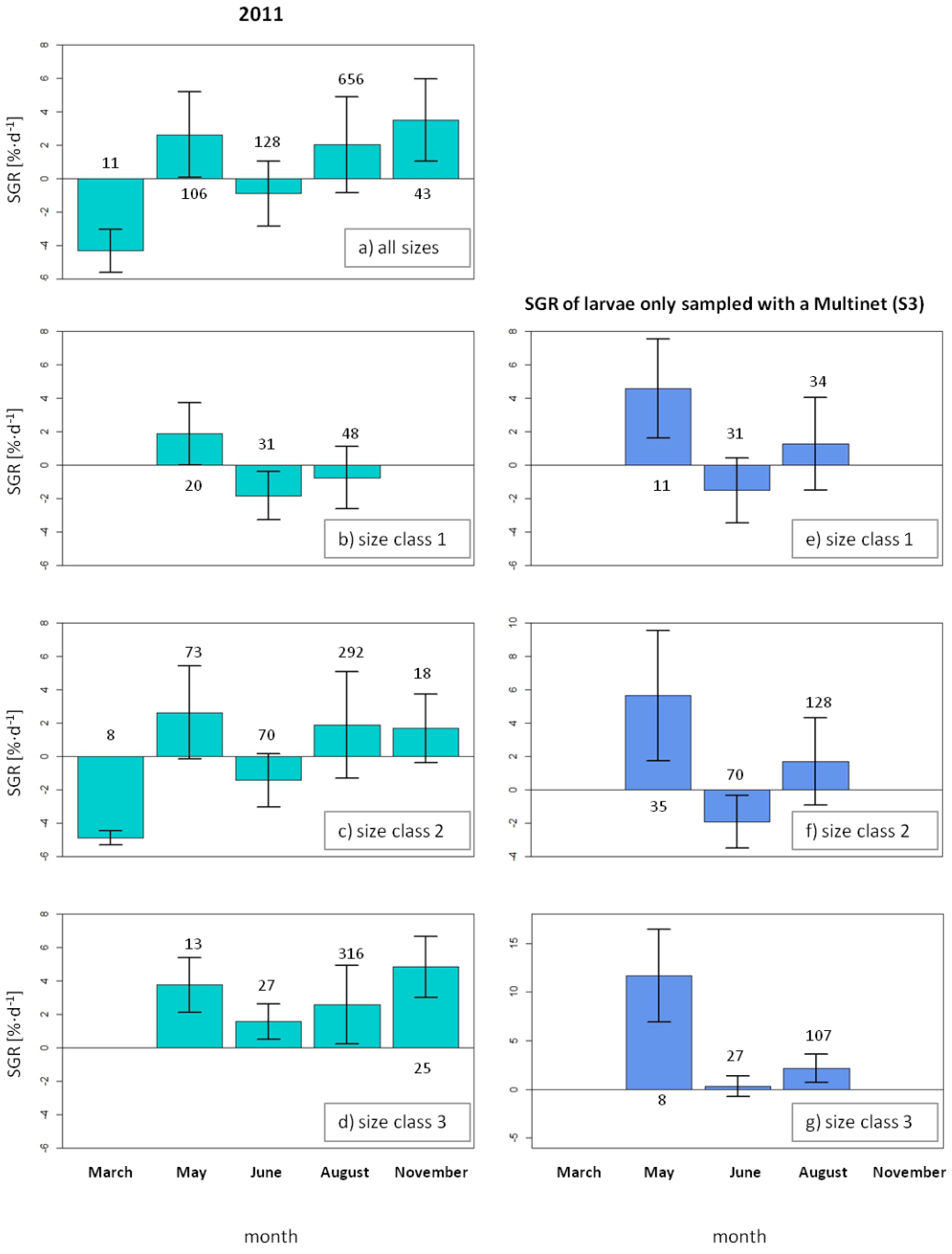

Fig. 10: Mean specific growth rates (SGR, %·d^{-1}, ±sd) of Baltic cod larvae caught in 2011. SGR is given over the season and sample sizes (N) are shown above or below the respective bar chart. Bar charts represent the mean SGR of all size classes (a), size class 1 (b), size class 2 (c) and size class 3 (d). Additional plots (e-g) represent the mean SGR (±sd) of Baltic cod larvae originating only from Multinet hauls using temperature of 5m depth intervals. (*Note:* Scaling of the y-axis of Fig. 10f and g differs from the other plots.)

Table 5: Monthly means (±sd) of specific growth rate in %·d^{-1} of Baltic cod larvae. Respective values are given for all sizes, size class1, 2 and 3.

Year	month	Size class	Mean ± sd	Year	month	Size class	Mean ± sd
2006	all	all sizes	0.35 ± 2.37	2011	all	all sizes	1.69 ± 2.98
	April		- 0.26 ± 2.38		March		- 4.33 ± 1.30
	June		0.66 ± 2.09		May		2.62 ± 2.56
	July		- 0.45 ± 1.74		June		- 0.91 ± 1.94
	November		3.49 ± 2.85		August		2.02 ± 2.86
					November		3.51 ± 2.47
	April	<4.5 mm	0.22 ± 2.47		March	< 4.5 mm	- 2.84± 1.77
	June		0.95 ± 3.41		May		1.87 ± 1.88
	July		- 1.33 ± 2.04		June		- 1.84 ± 1.45
	November		1.38 ± 4.08		August		- 0.76 ± 1.87
	June	4.5 to 6 mm	- 0.20 ± 1.19		March	4.5 to 6 mm	- 4.90 ± 0.44
	July		- 0.89 ± 1.35		May		2.67 ± 2.79
	November		1.58 ± 2.35		June		- 1.45 ± 1.59
					August		1.89 ± 3.21
					November		1.68 ± 2.06
	June	> 6 mm	1.30 ± 1.20		May	> 6 mm	3.76 ± 1.64
	July		0.54 ± 1.44		June		1.57 ± 1.06
	November		4.73 ± 1.91		August		2.58 ± 2.34
					November		4.83 ± 1.83

4.5 Growth performance of Baltic cod larvae

In order to ecologically evaluate the specific growth rates (SGRs) of Baltic cod larvae, individual data of observed growth rates were compared to reference growth rates (G_{ref}) of Atlantic cod (*Gadus morhua*), predicted by the size-temperature-dependent growth model (STDG model) developed by Folkvord (2005), and resulting in the so-called growth performance (G_{pf}).

Mean growth performance and the standard deviation for the different years and months are given in Table 6. Fig. 11-14 present the comparison between SGR and G_{ref} and the consequent G_{pf} for I) the years 2006, 2007 (only August) and 2011 and II) for months with highest nutritional condition (sRD) in 2006 and 2011 and highest sample size in August 2011. Results of a multi-factorial analysis of variance displayed significant influence of the factors year and month on the growth performance of Baltic cod larvae (Table 7). The observed growth of Eastern Baltic cod larvae in 2006, 2007 and 2011 showed deviations from the predicted maximum growth rates, with G_{ref} presenting overall higher growth rates than actually achieved by cod larvae from the Bornholm Basin (Fig. 11, Fig. 13),. In 2006 and 2007 differences were more pronounced than in 2011 (Fig. 11) resulting in growth performances of Baltic cod larvae mainly ranging from -1.03 to 0.58 in 2006, -0.45 to 0.50 in 2007 (only August) and -0.68 to 1.86 over the year 2011 (Fig. 12). The year 2011 displayed highest mean G_{pf} of Baltic cod larvae over the season.

Considering the presented months, June 2006 showed strongest differences between the predicted and observed growth rates (Fig. 13a and d). The months May and August 2011 showed higher approximation between SGR and G_{ref} (Fig. 12b and e; c and f). In June 2006 the percentage of larvae with G_{pf} <0, from 0 to 0.5 and >0.5 was 29%, 71% and 0% respectively (Fig. 14). May 2011 showed highest proportion of larvae with a G_{pf} >0.5 (6%, 28% and 66% for G_{pf} <0, 0 to 0.5 and >0.5, respectively). Larvae with a growth performance from 0 to 0.5 were most dominant in August 2011 (12%, 72% and 16% for G_{pf} <0, 0 to 0.5 and >0.5, respectively). The illustration of growth performance of Baltic cod larvae showed highest variances for larvae with a dry weight up to about 125 µg (Fig. 12, Fig. 14). The mean G_{pf} between the size classes 2 and 3 displayed higher mean values for larvae > 6mm.

Table 6: Monthly means (±sd) of growth performance (G_{pf}, dimensionless) of Baltic cod larvae (*Gadus morhua*) in 2006, August 2007 and 2011. By multiplying the G_{pf} with 100 one would obtain the percentage of actual growth realized by larvae in relation to the reference growth rate (G_{ref}) predicted by the literature for larvae growing at maximum rates at given size and temperature.

Year	month	Size class	Mean ± sd	Year	month	Size class	Mean ± sd
2006	June	all sizes	0.03 ± 0.24	2011	May	all sizes	0.71 ± 0.47
	July		0.06 ± 0.25		June		0.23 ± 0.33
	November		0.28 ± 0.14		August		0.28 ± 0.28
					November		0.36 ± 0.20
	June	4.5 to 6 mm	- 0.13 ± 0.26		May	4.5 to 6 mm	0.67 ± 0.16
	July		- 0.18 ± 0.30		June		0.16 ± 0.71
	November		0.18 ± 0.19		August		0.30 ± 0.42
					November		0.22 ± 0.29
	June	> 6 mm	0.14 ± 0.16		May	> 6 mm	0.77 ± 0.44
	July		0.03 ± 0.15		June		0.24 ± 0.25
	November		0.30 ± 0.12		August		0.27 ± 0.23
					November		0.41 ± 0.13
2007	August	all	0.09 ± 0.18				

Table 7: Summary of outcome of the multifactorial analyses of variance for the standardized R/D (sRD), specific growth rate (SGR), and growth performance (G_{pf}) of cod larvae.

	factor	Df	F value	P
sRD	year	1	14.60	< 0.001***
	month	6	76.65	< 0.001***
	size class	2	172.45	< 0.001***
	year*month	1	29.43	< 0.001***
	year*size class	2	0.18	0.8 (n.s.)
	month*size class	11	4.90	< 0.001***
	year*month*size class	1	2.78	0.1 (n.s)
SGR	year	1	43.91	< 0.001***
	month	6	98.99	< 0.001***
	size class	2	84.78	< 0.001***
	year*month	1	6.98	0.008**
	year*size class	2	4.69	0.009**
	month*size class	11	7.97	< 0.001***
	year*month*size class	1	1.70	0.2 (n.s.)
Gpf	year	2	41.34	<0.001***
	month	5	16.51	<0.001***
	size class	2	1.94	0.1 (n.s.)
	year*month	1	0.82	0.4 (n.s.)
	year*size class	1	0.25	0.6 (n.s.)
	month*size class	4	1.87	0.1(n.s.)
	year*month*size class	1	0.53	0.5 (n.s.)

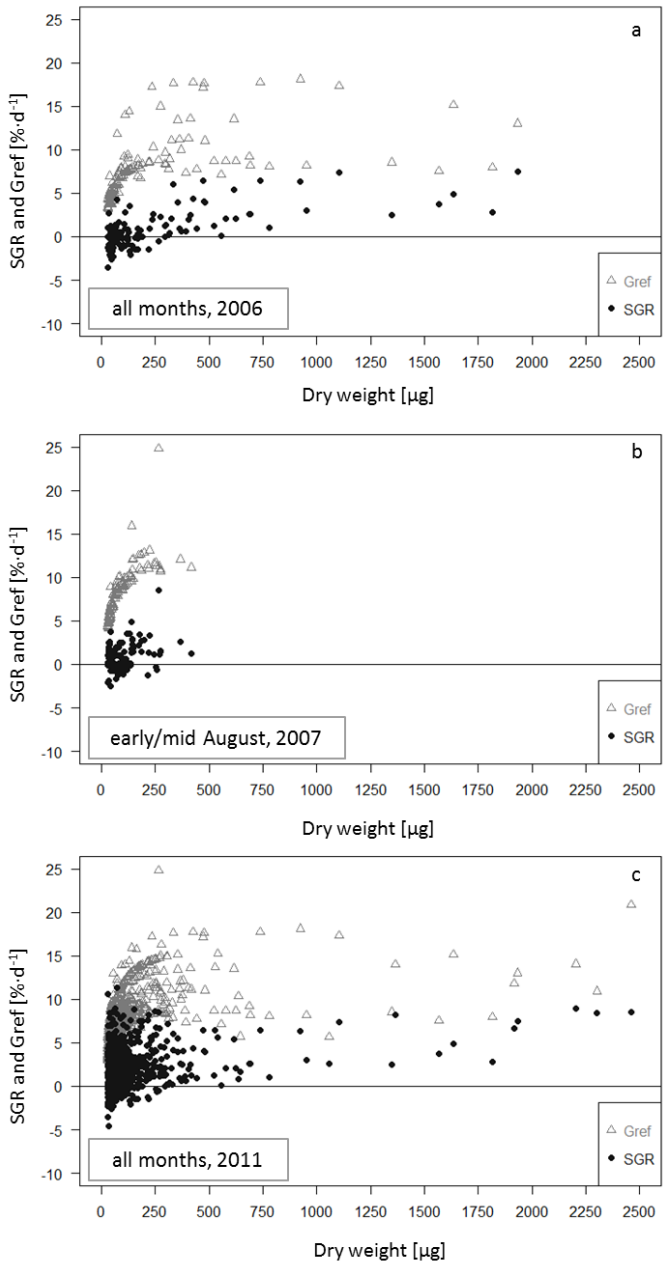

Fig. 11: Observed specific growth rates (SGR) of individual Baltic cod larvae (black circles) and predicted growth rates for larvae growing at maximum potential (grey triangles) plotted over dry weight (µg) for the years 2006, 2007 and 2011. Predicted growth rates were estimated using the STDG model by Folkvord (2005) with given larval dry weights and temperatures of the Baltic cod larvae analysed in this thesis. Larvae with a DW <30 µg were excluded from the analyses, as coverage by the STDG model is not given. Also modeled reference growth rates with negative values and the respective SGRs were ignored.

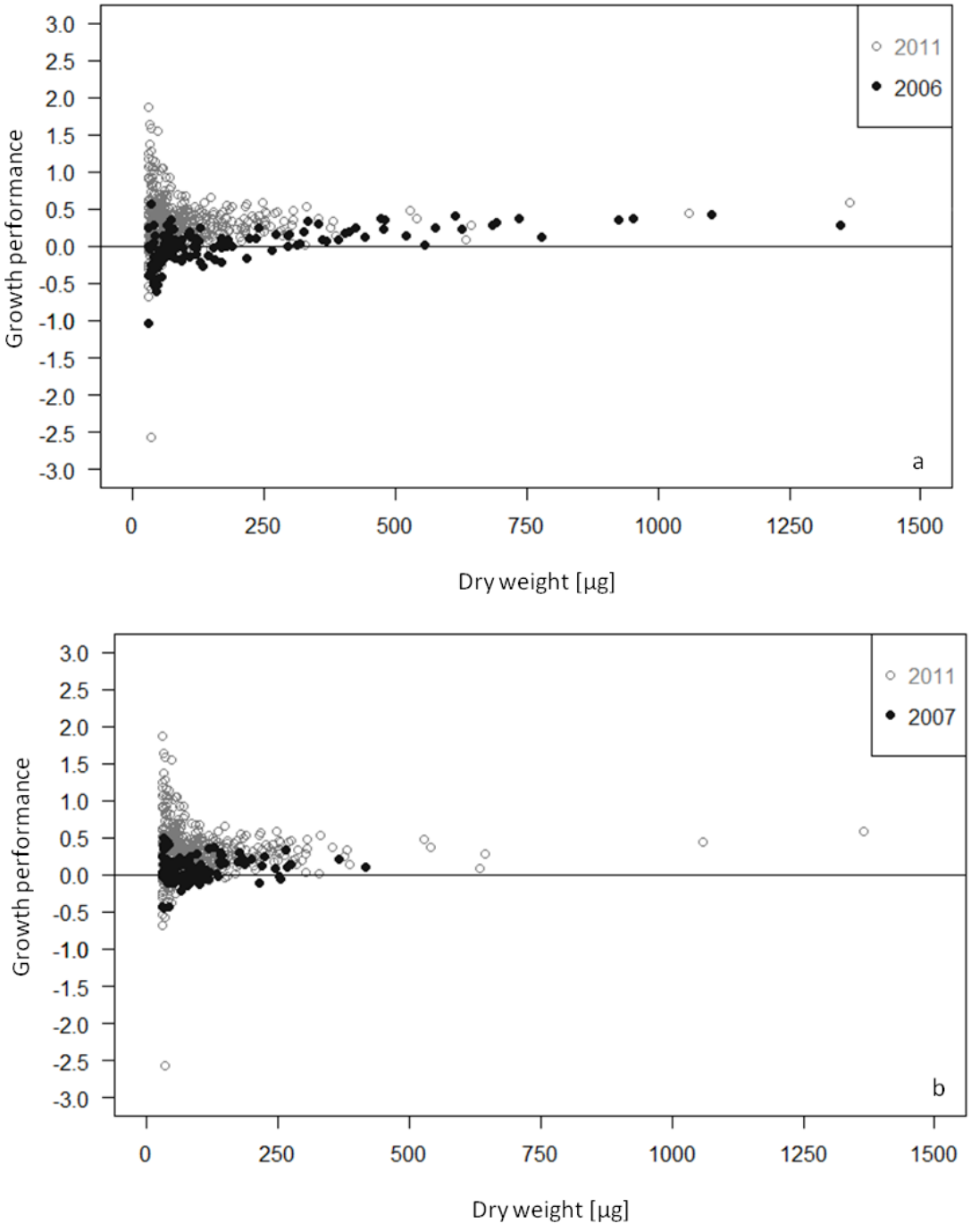

Fig. 12: Growth performance (G_{pf}) over dry weight (µg) of Baltic cod larvae between the years a) 2006 (black) and 2011 (grey) and b) 2007 (black) and 2011 (grey).

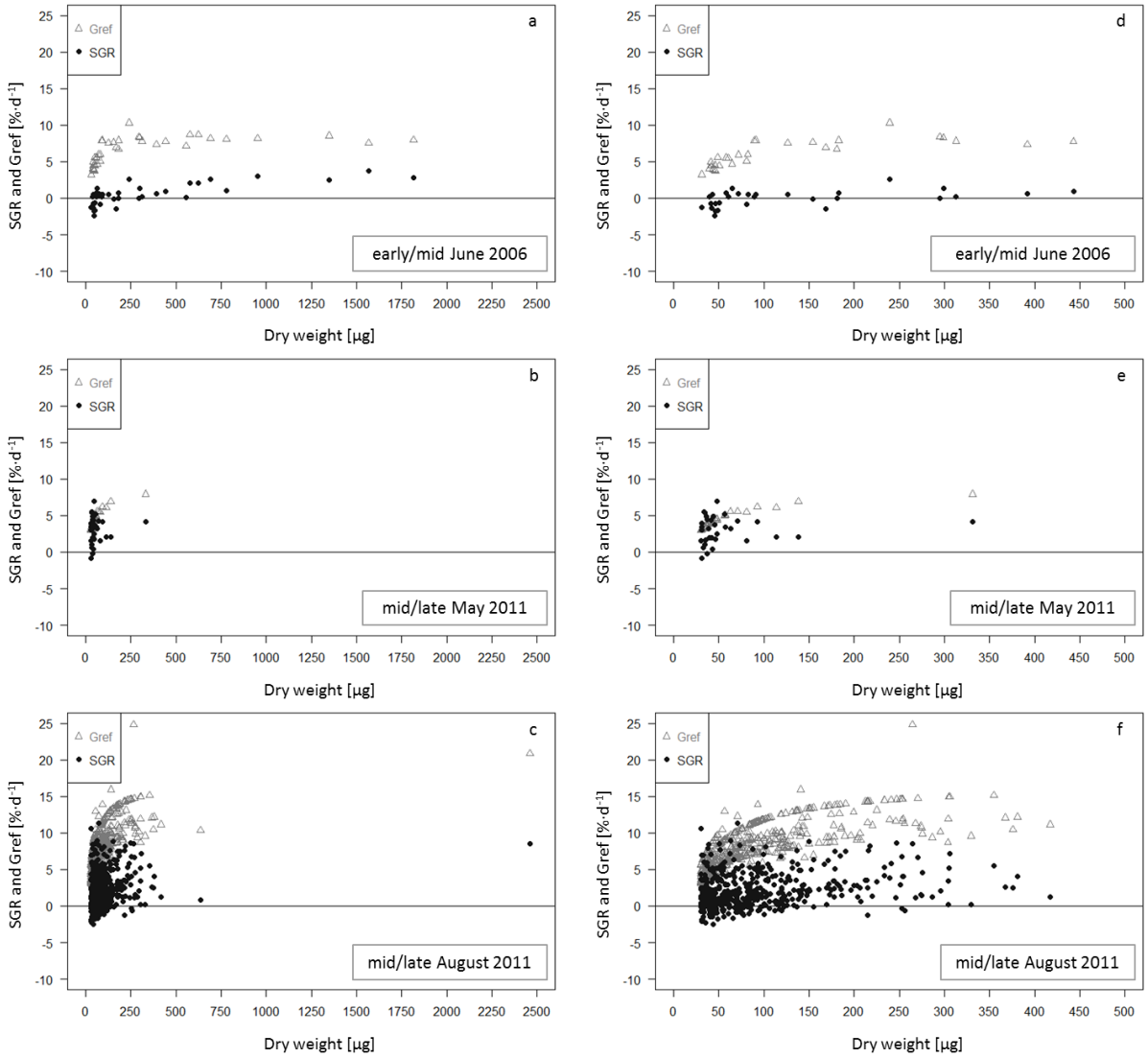

Fig. 13: Observed specific growth rates (SGR) of individual Baltic cod larvae (green triangles) and predicted growth rates for larvae growing at maximum speed (red circles) plotted over dry weight (DW, µg) for the months June 2006 and May and August 2011. Respective months were chosen based on highest larval nutritional condition (sRD, June 2006 and May 2011) or highest larval abundance (August 2011). Predicted growth rates were estimated using the STDG model by Folkvord (2005) with given larval dry weights and temperatures of the Baltic cod larvae analysed in this thesis. Larvae with a DW <30 µg were excluded from the analyses, as coverage by the STDG model is not given. Also modeled reference growth rates with negative values and the respective SGRs were ignored. Left panel presents the full range of cod larvae dry weights determined in the study, the right panel shows a selected range up to 500 µg dry weight.

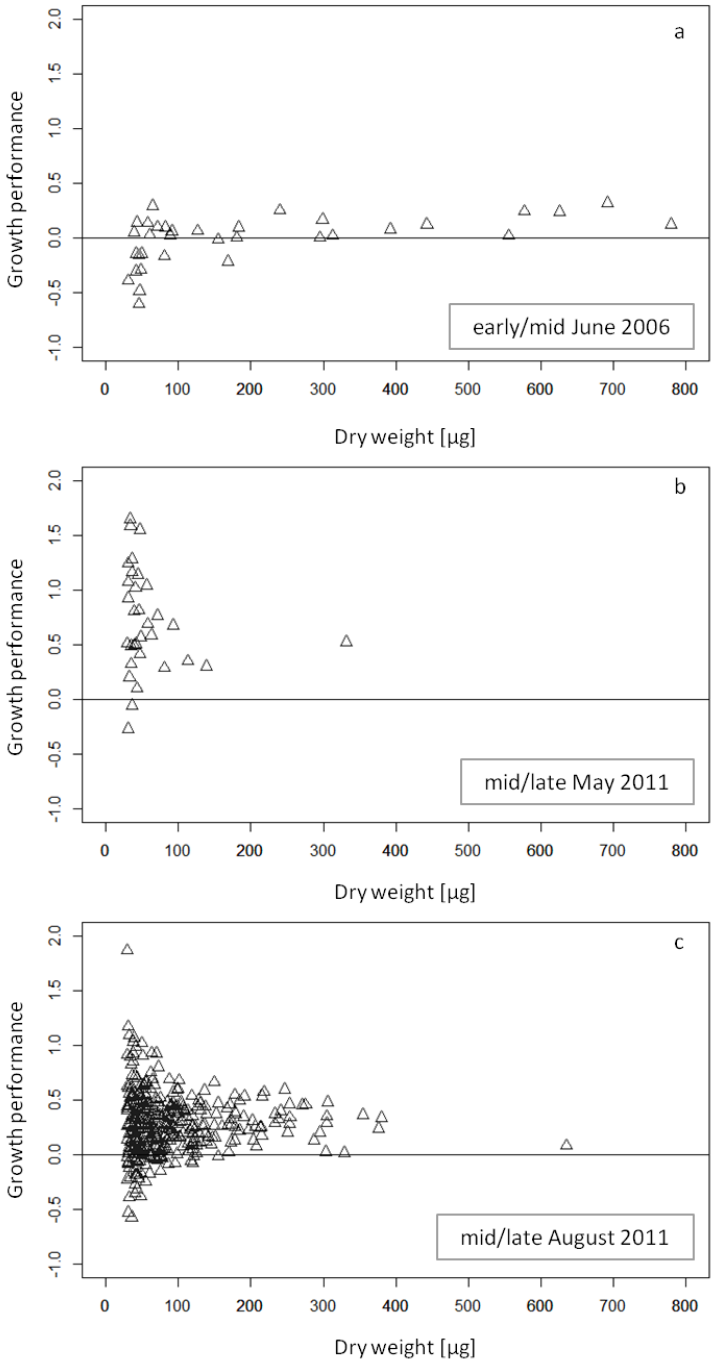

Fig. 14: Growth performance (G_{pf}) of larval Baltic cod over dry weight (DW, µg). G_{pf} is given for a) June 2006, b) May 2011 and c) August 2011. Larvae with a DW <30 µg were excluded from the analyses, as coverage by the STDG model is not given. Also for modeled reference growth rates with a negative value no estimation of G_{pf} was performed.

4.6 Suitable prey fields and correlation with nutritional condition of Baltic cod larvae

The seasonal occurrence of larval prey in terms of abundance and biomass is shown for the years 2006 and 2011 (Fig. 15). Both abundance and biomass of suitable prey items were also correlated with nutritional condition of analysed Baltic cod larvae divided into functional size groups (Fig. 16 to Fig. 21). Based on the results of a Pearson´s product-moment correlation matrix larval nutritional condition was correlated with I) all copepod species (N+C1), II) only *Pseudocalanus* spp. (N+C1), III) all other copepod species, and IV) species of the rotifer genera. Additional correlations are displayed for the cumulative occurrence of I) all copepod species and rotifers and II) the species *Pseudocalanus* spp. and rotifers. As exemplarily shown in Table 8, the extension of prey fields with further developed stages of *Pseudocalanus* spp. reduced the correlation between larval nutritional condition and abundance/biomass of this species. This was also true for all copepod species.

Table 8: Pearson´s product-moment correlation between mean values of larval cod standardized R/D ratios and different functional groups of suitable developmental stages of the copepod species *Pseudocalanus* spp.in relation to abundance and biomass.

Functional prey group	**larval size class**	**abundance**	**biomass**
Nauplii and C1	< 4.5 mm	0.79	0.90
Nauplii and C1 - C2		0.74	0.50
Nauplii and C1 - C3		0.46	0.20
Nauplii and C1	4.5 to 6 mm	0.91	0.79
Nauplii and C1 - C2		0.59	0.26
Nauplii and C1 - C3		0.37	0.04
Nauplii and C1	> 6 mm	0.88	0.81
Nauplii and C1 - C2		0.64	0.35
Nauplii and C1 - C3		0.14	-0.19

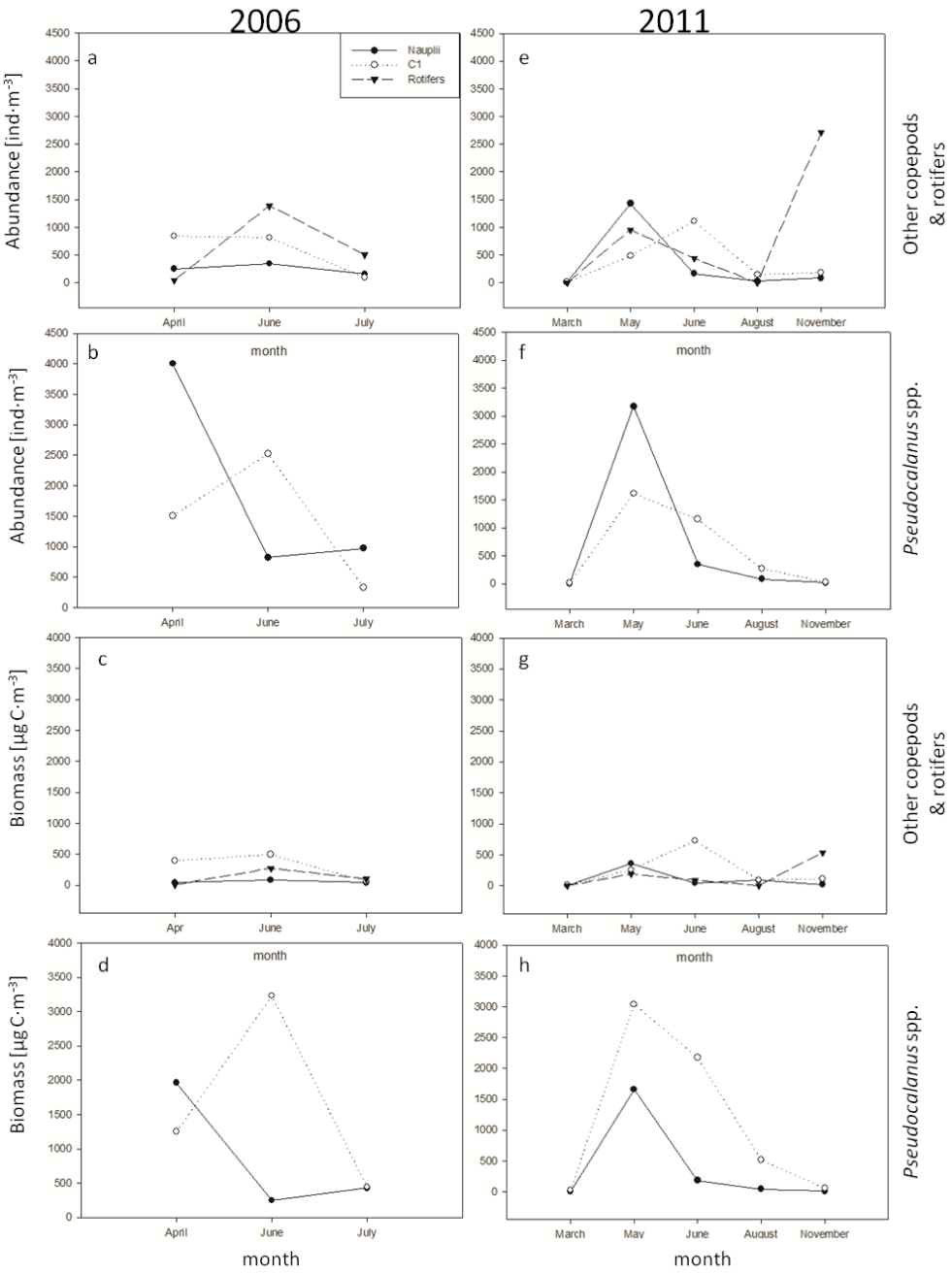

Fig. 15: Seasonal abundance (individuals·m^{-3}) and biomass (µg C·m^{-3}) of early developmental stages of copepod species and rotifers occurring in the Bornholm Basin in 2006 (left panel) and 2011 (right panel). Plots for "other copepods" show copepod species including *Acartia* spp., *Oithona similis*, *Centropages hamartus*, and *Temora longicorni*s. Plots labelled with "*Pseudocalanus* spp." present only the species *Pseudocalanus* spp. The rotifer genera is included in all graphs.

In 2006, mean abundance and biomass of copepodite developmental stages and rotifers varied between the months (Fig. 15, left panel). Nauplii stages of other copepod species displayed both, highest abundance and biomass in June, lowest in July (Fig. 15a and c). The species *Pseudocalanus* spp. presented a peak in nauplii abundance and biomass in April and lowest mean values in June (Fig. 15b and d). The C1 stages of all other copepods showed highest mean abundance in April and highest biomass in June 2006 (Fig. 15a and c). For the species *Pseudocalanus* spp. only, a peak in abundance and biomass could be observed in June (Fig. 15b and d). The emergence of the rotifer genera was characterized by a peak in abundance and biomass during June and lowest values in April.

The seasonal occurrence of copepod nauplii, copepodite stage 1 and the rotifer genera in 2011 presented differences between the months (Fig. 15e-h, right panel). Nauplii of all other copepod species and *Pseudocalanus* spp. alone peaked in abundance and biomass in May (Fig. 15e-h). While C1 stages of the species *Pseudocalanus* spp. peaked in abundance and biomass in May (Fig. 15f and h), all other copepods showed highest values in June (Fig. 15e and g). Specimens of the rotifer genera presented increased mean values in May and peaked in abundance and biomass in November (Fig. 15e-h).

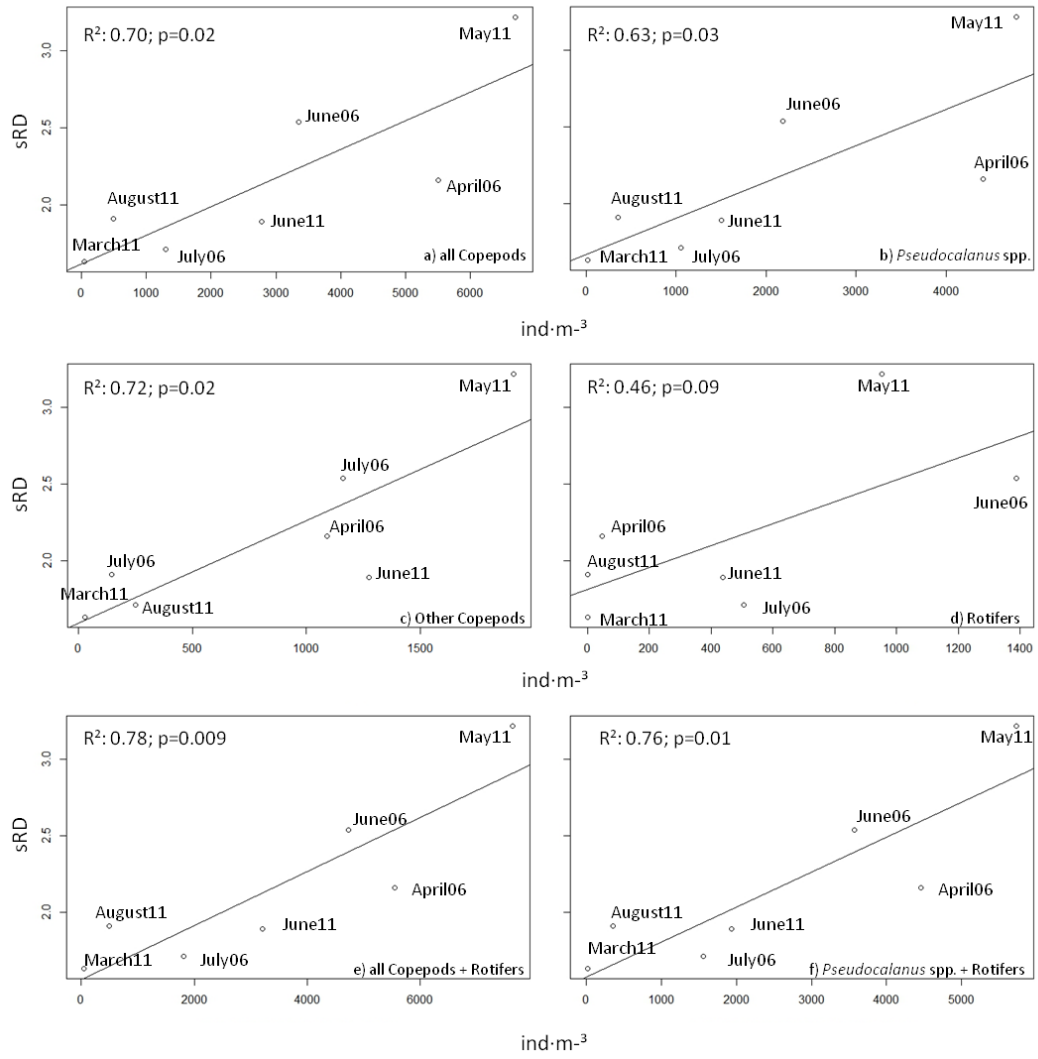

Fig. 16: Relation between nutritional condition of Baltic cod larvae and abundance of suitable prey fields for **larvae < 4.5 mm**. Plots are given for sRD as a function of **abundance** of a) all copepod species, b) *Pseudocalanus* spp., c) other copepod species, d) rotifers, e) the cumulative abundance of all copepods and rotifers and f) *Pseudocalanaus* spp. and rotifers. Data are mean values of sRD and food availability for the different months, correlation coefficients (R^2) and p-values are presented.

For larvae < 4.5 mm (sc1) highest correlation between standardized R/D ratio (sRD) and the abundance of suitable prey was shown for the combination of all available copepod species and rotifers (Fig. 16d; R^2=0.78; p=0.009). Individual correlations for all copepod species, *Pseudocalanus* spp. alone, all other copepods and the rotifer genera showed highest correlation coefficients for all other copepods (Fig. 16c).

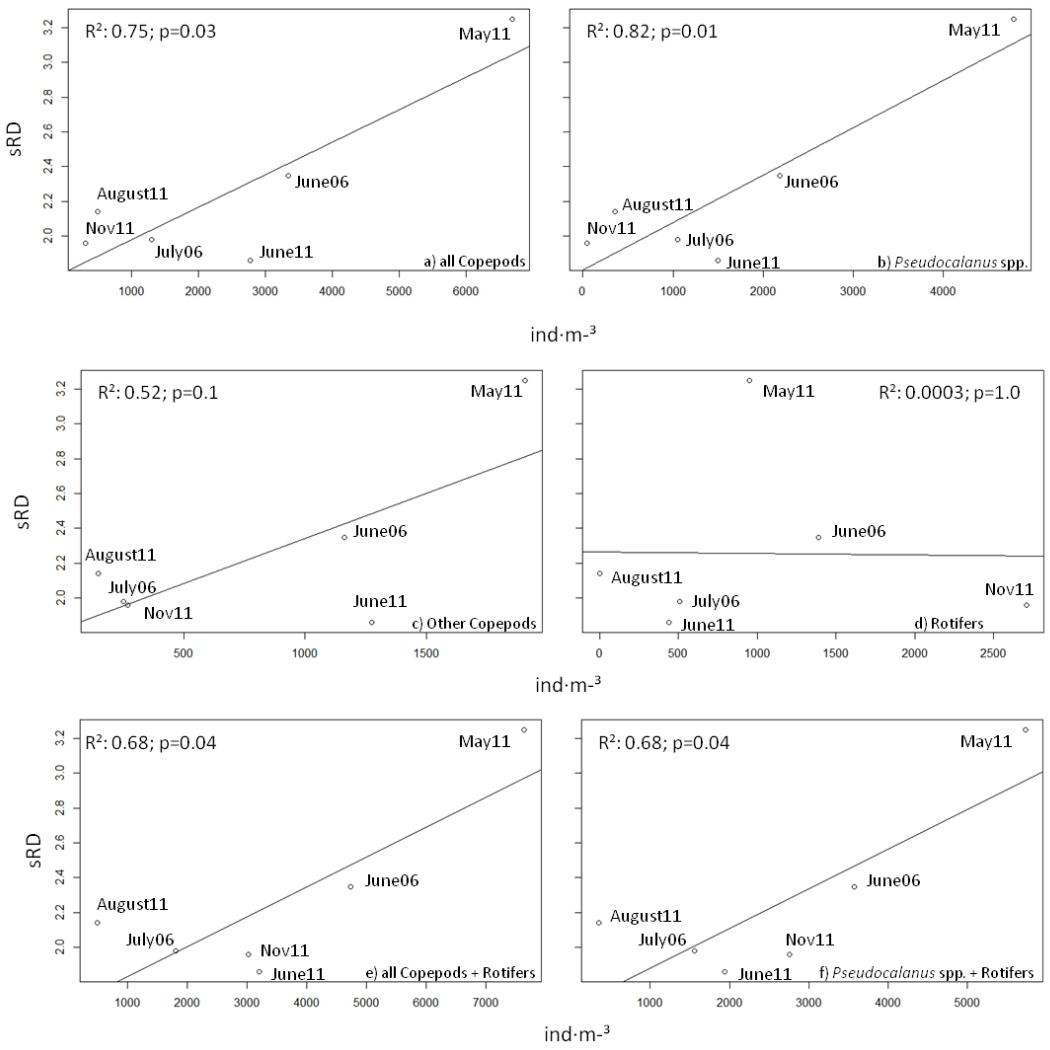

Fig. 17: Relation between nutritional condition of Baltic cod larvae and abundance of suitable prey fields for **larvae** from **4.5 to 6 mm**. Plots are given for sRD as a function of **abundance** of a) all copepod species, b) *Pseudocalanus* spp., c) other copepod species, d) rotifers, e) the cumulative abundance of all copepods and rotifers and f) *Pseudocalanaus* spp. and rotifers. Data are mean values of sRD and food availability for the different months, correlation coefficients (R^2) and p-values are presented.

For size class 2 (4.5 to 6 mm) highest explanation for the larval nutritional condition (sRD) was presented by the abundance of *Pseudocalanus* spp. (Fig. 17e; R^2=0.82, p=0.01). The combination with other copepod species and rotifers reduced the explanatory power of the correlation.

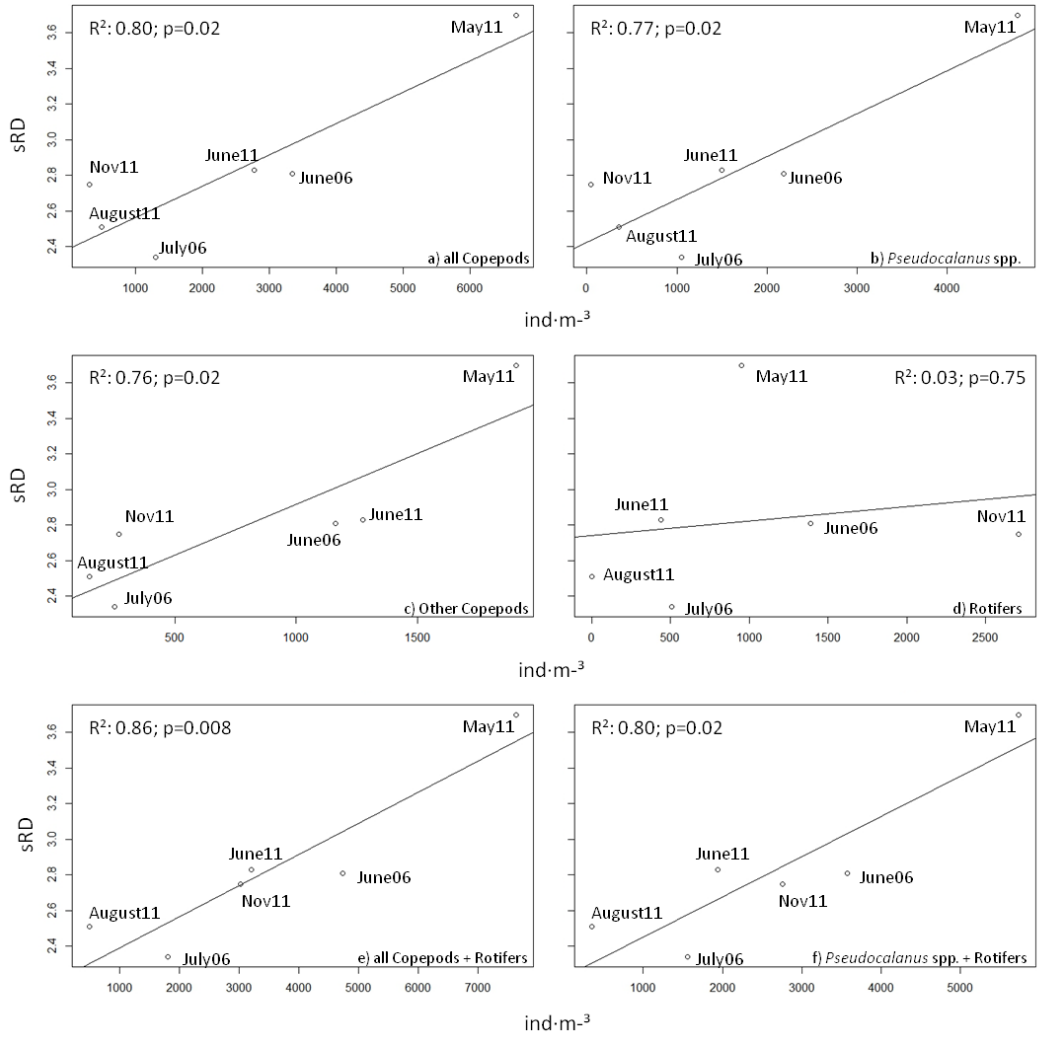

Fig. 18: Relation between nutritional condition of Baltic cod larvae and abundance of suitable prey fields for **larvae > 6 mm**. Plots are given for sRD as a function of **abundance** of a) all copepod species, b) *Pseudocalanus* spp., c) other copepod species, d) rotifers, e) the cumulative abundance of all copepods and rotifers and f) *Pseudocalanaus* spp. and rotifers. Data are mean values of sRD and food availability for the different months, correlation coefficients (R^2) and p-values are presented.

The mean sRDs of size class 3 of Baltic cod larvae analysed in this study (> 6mm) presented highest correlation with the combined abundance of all copepod species and rotifer species (Fig. 18d; R^2=0.86, p=0.008). Individual correlation showed highest explanatory power by all copepod species (Fig. 18a).

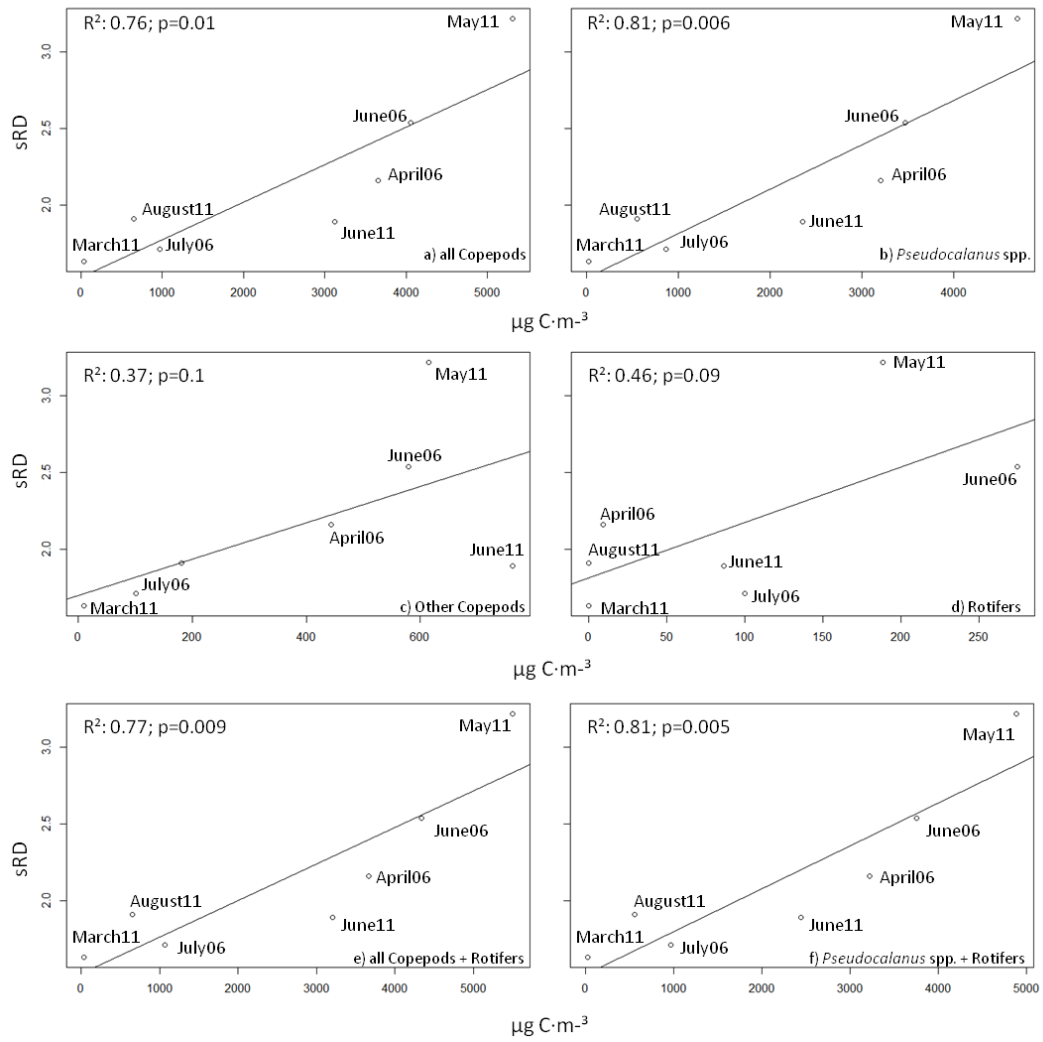

Fig. 19: Relation between nutritional condition of Baltic cod **larvae < 4.5 mm** and the **biomass** of suitable prey fields occurring in the Bornholm Basin. Plots are given for sRD as a function of biomass of a) all copepod species, b) *Pseudocalanus* spp., c) other copepod species, d) rotifers, e) the cumulative abundance of all copepods and rotifers and f) *Pseudocalanaus* spp. and rotifers. Data are mean values of sRD and food availability for the different months, correlation coefficients (R^2) and p-values are presented.

In terms of correlating biomass of suitable prey fields with the sRD of Baltic cod larvae of sc1, the combination of rotifers and *Pseudocalanus* spp. showed highest explanatory power (Fig. 19e; R^2=0.81, p=0.005), while the inclusion of rotifers slightly increased the significance but not the explained variance (Fig. 19b and e). Inclusion of all copepod species led to a reduced correlation (Fig. 19a and e).

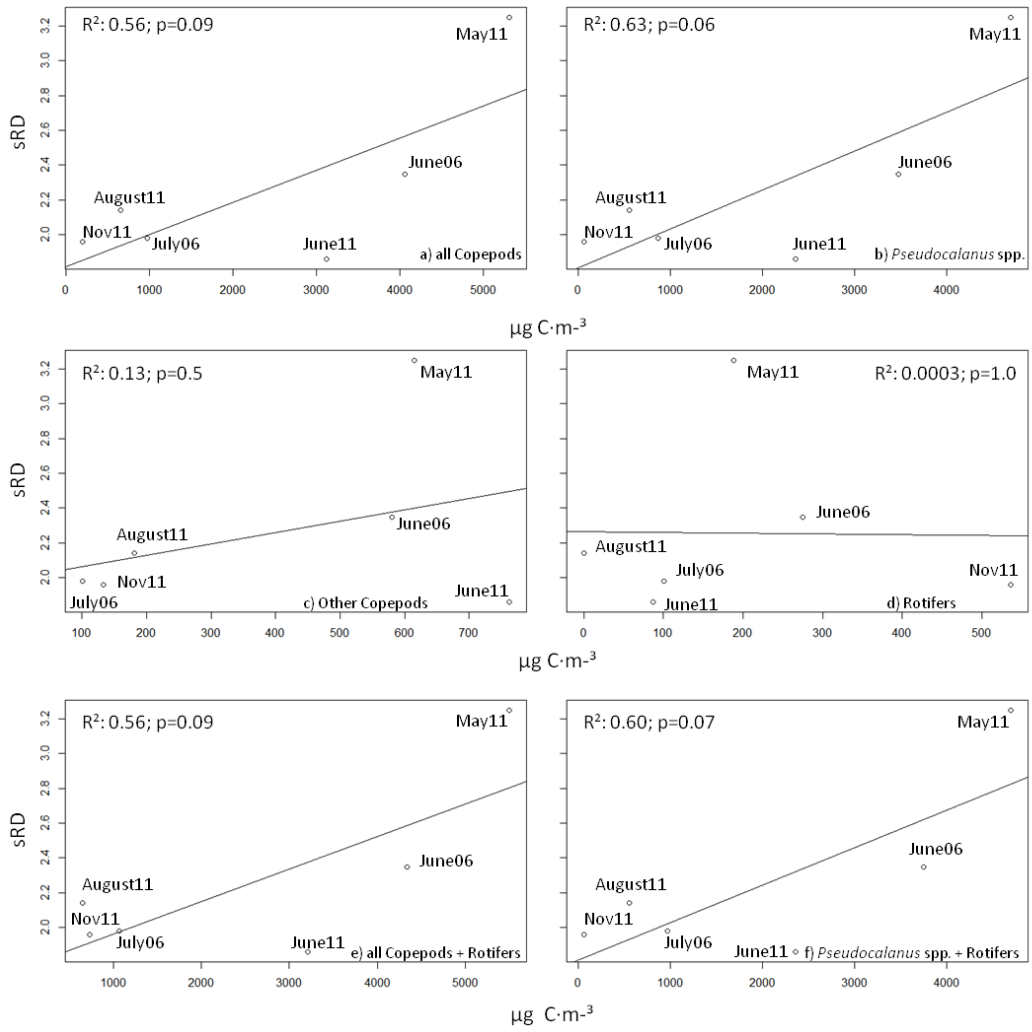

Fig. 20: Relation between nutritional condition (sRD) of Baltic cod **larvae** from **4.5 to 6 mm** and the **biomass** of suitable prey fields occurring in the Bornholm Basin. Plots are given for sRD as a function of biomass of a) all copepod species, b) *Pseudocalanus* spp., c) other copepod species, d) rotifers, e) the cumulative abundance of all copepods and rotifers and f) *Pseudocalanaus* spp. and rotifers. Data are mean values of sRD and food availability for the different months, correlation coefficients (R^2) and p-values are presented.

The mean sRD of larvae from size class 2 showed highest correlation in combination with the mean biomass of *Pseudocalanus* spp. (Fig. 20b; R^2=0.63, p=0.06). Further inclusion of other copepod species and rotifers reduced the explanatory power of the correlation (R^2 from 0.56 to 0.60).

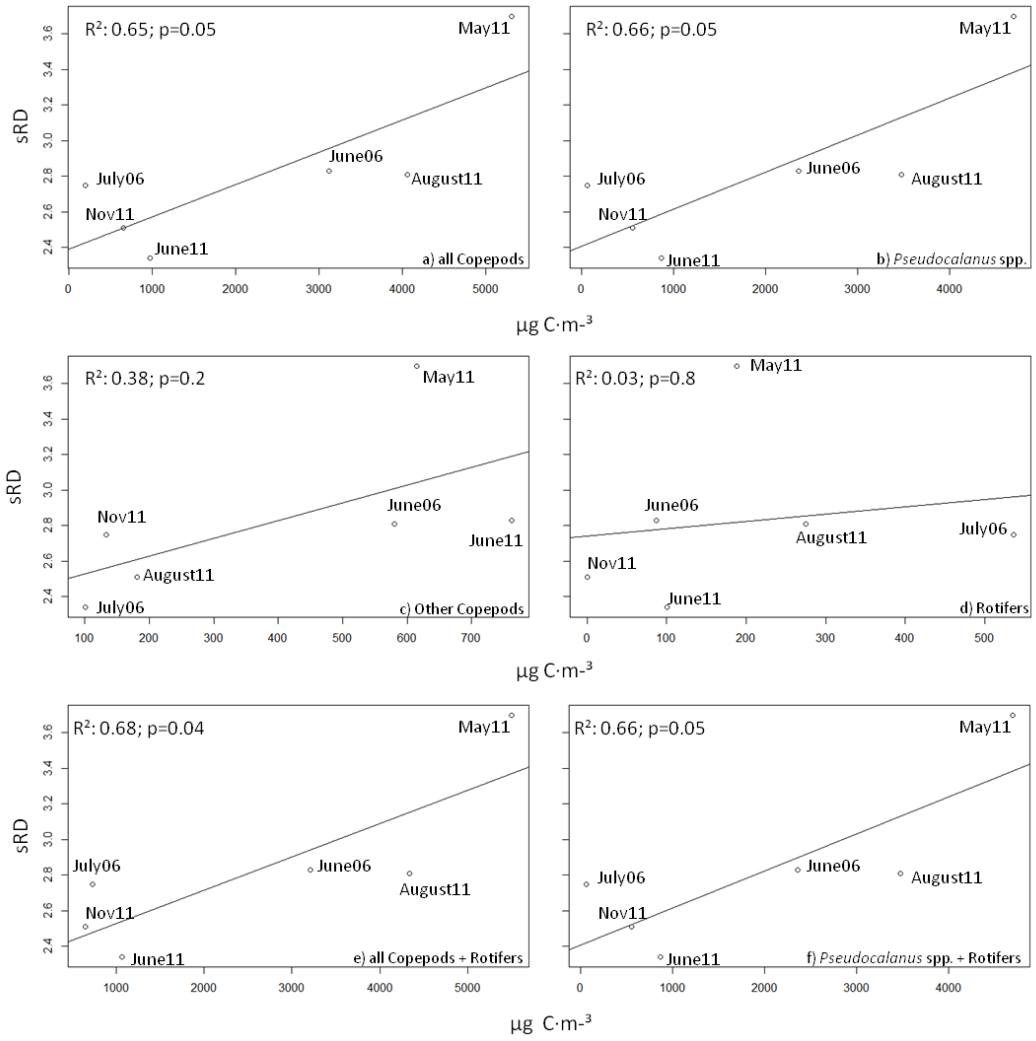

Fig. 21: Relation between nutritional condition of Baltic cod **larvae > 6 mm** and the **biomass** of suitable prey fields occurring in the Bornholm Basin. Plots are given for sRD as a function of biomass of a) all copepod species, b) *Pseudocalanus* spp., c) other copepod species, d) rotifers, e) the cumulative abundance of all copepods and rotifers and f) *Pseudocalanaus* spp. and rotifers. Data are mean values of sRD and food availability for the different months, correlation coefficients (R^2) and p-values are presented.

The mean sRDs of Baltic cod larvae of sc3 displayed highest correlation with the combined biomass of all copepod species and rotifers (Fig. 21d; R^2=0.68, p=0.04). By individual correlation the species *Pseudocalanus* spp. showed the highest explanatory power of 66% (Fig. 21b; p=0.05).

5 Discussion

5.1 Abundance and length-frequency of Baltic cod larvae

The abundance of larvae sampled for biochemical analyses differed extremely between the seasons 2006 and 2011. Despite assessments by ICES (2013) predicting poor abiotic conditions and hence a low "reproductive volume" (RV) for Eastern Baltic cod in the year 2011, abundances of larvae in 2011 were highest since the 1980s and considerably higher than in the period from the late 1980's to 2010, with the exception of 2003 which also showed relatively high, but still lower abundance as 2011 (Köster *et al.* unpubl.). High larval abundances may indicate high egg survival, hatching rates and survival of cod larvae in 2011 as the abundance of cod eggs in 2011 was relatively low compared to other years, including 2006 and 2007 which were also analysed in the present study (Köster *et al.* unpubl., Bastian Huwer, pers. comm.).

The analysis of the length-frequency distribution of larvae within a season could provide information on the potential growth and survival success of different cohorts depending on the time of hatching. However, the tracking of consecutive cohorts over the season based only on monthly length frequency distributions was not possible, even though the mean larval size increased over the seasons. Despite indications of a slight back-shift towards historically found peak spawning time of Baltic cod (MacKenzie *et al.*1996, Schaber *et al.* 2011), the highest abundance of sampled cod larvae in July 2006 and August 2011 supports the suggestion that spawning of the Eastern Baltic cod stock peaks during June-July (Wieland 2000). However, inter-annual fluctuations in peak spawning time have been reported for the Eastern Baltic cod stock (Wieland 2000, Bleil *et al.* 2009).

5.2 Nutritional condition, growth rate and growth performance of Baltic cod larvae

The comparison between the years 2006, 2007 and 2011 presented the nutritional condition (sRD), growth rate (SGR) and growth performance (G_{pf}) of Baltic cod larvae to be significantly affected by the factors year and month. Further, the size class of cod larvae had a significant effect on sRD and SGR. Overall, better nutritional condition, growth rates and growth performance was found in the year 2011. Therefore the proposed null hypothesis $H_0$1 (*Nutritional condition and growth of Baltic cod larvae is consistent between the years.*) could be rejected. Nutritional condition and growth also showed seasonal variability, indicating the importance of the timing of larval emergence with the occurrence of advantageous biotic and abiotic environmental conditions. Hence, the $H_0$2 (*Nutritional condition and growth of Baltic cod larvae is consistent within the spawning season.*) could also be rejected.

5.2.1 Nutritional condition

In general, sRD ratios in cod larvae observed in the present study, ranged between 1.4 and 3.7 showing lower ratio values than usually reported for Atlantic cod in the literature (e.g. Caldarone *et al.* 2003, Buckley *et al.* 2006).

By focusing on the different larval size classes the present study revealed, that the nutritional condition during the early life history of cod larvae changes with respect to larval standard length. Larvae < 4.5 mm (sc1) presented overall lowest sRD ratios with nutritionally better conditioned larvae occurring during the early season (2006: April-June; 2011: May). Considering sc1 to be pre-feeding larvae, the better nutritional condition earlier in the year might be explained by a maternal effect as more viable eggs are assumed to be spawned by older (≥ 5 years) and bigger females early in the season (Vallin and Nissling 2000, Buckley *et al.* 2010). Another explanation comes with the assumption, that Baltic cod larvae < 4.5 mm already initiated the transition from endo- to exogenous nutrition and therefore presenting variability in nutritional condition potentially influenced by changes in food availability. Despite of laboratory derived findings of initially decreased R/D ratios after the yolk sac phase (e.g. Clemmesen 1994, Caldarone 2005), cod larvae from 4.5 to 6 mm (first feeding) presented slightly higher sRD ratios compared to smaller larvae, potentially supporting the suggestion, that attempts of first feeding in Baltic cod larvae from this study already started at standard lengths < 4.5 mm. The

nutritional condition of larvae from 4.5 to 6 mm was found to be highest in June 2006 and May 2011. The biggest larvae investigated in this study ranging from > 6 to a maximum larval standard length of 15 mm displayed the highest ratios of sRD. Differences in the sRD ratios between months again showed cod larvae with best nutritional condition in June 2006 and May 2011 indicating the importance of suitable prey fields as energy resource early in the season, which has also been suggested for sprat (*Sprattus sprattus*) larvae in the Baltic Sea (Voss *et al.* 2006).

5.2.2 Growth rate

The estimation of growth rates based on the multi-species model developed by Buckley *et al.* (2008) revealed inter-annual and inter-seasonal variability, mainly driven by ambient temperature and nutritional condition of larvae. While in both years the temperatures earlier in the spawning season were lower and increased over the season, the best nutritional condition of larvae was found in June 2006 and May 2011. Also the growth rates of larvae belonging to the size classes 1 and 2 were found to be highest in the respective months. The size class 3 showed highest growth rates in November during both years, followed by growth rates in months of highest sRD in June 2006 and May 2011, respectively. Relatively good nutritional condition and warmer temperatures in deeper water masses due to a reduced thermocline in November particularly seemed to benefit growth rates of larvae > 6 mm, also indicating that cod larvae encountered suitable prey fields late in the season. The overall high proportion of larvae < 4.5 mm (depth range 50 – 70 m) with negative growth rates, is in accordance with findings of Grønkjær *et al.* (1997), who found Baltic cod larvae to grow positively only in samples from depths shallower than 45 m. Hence, the hypothesis of an obligatory ontogenetic migration of first feeding cod larvae from hatching depths to shallower water layers in the Bornholm Basin (Grønkjær *et al.* 1997, Huwer *et al.* 2011) is supported by the present study.

The additional estimation of growth rates for larvae only originating from Multinet hauls in 2011 allowed for a more accurate attribution of temperature values recently experienced by a larva. The results for the temperature use of the respective 5m depth intervals, presented even higher growth rates of all larvae independent of size class during May 2011. Mean growth rates of sc1 in August 2011 increased from negative to positive SGRs. For larvae of sc2 and sc3, mean

growth rates slightly decreased in June and August 2011, with highest reduction in SGR for larvae of sc3 in June (1.45 $\%\cdot d^{-1}$ less growth). Still, the application of uniformly calculated temperature means over defined depth ranges with respect to larval size can be considered a conservative approach, as it seemed to produce similar or even underestimated larval growth rates when compared to those obtained by Multinet temperatures.

Either way, growth rates of Baltic cod larvae have been found to be low over the range of size classes when compared to cod larvae from other areas (e.g. Buckley *et al.* 2004). Huwer *et al.* 2011 already discussed that for the Baltic ecosystem, where suitable larval food became limited (Möllmann *et al.* 2000) and predators on larvae are scarce due to a small vertical overlap (Köster and Möllmann 2000), not a maximum-growth but rather a slow-growth strategy may favour survival due to higher starvation resistance (Bochdansky *et al.* 2005). This study supports the suggestion that within the Bornholm Basin slow-growing larvae have higher potential to stay in the system compared to ecosystems with high predation pressure, potentially even being favoured under conditions of low temperatures and limited food availability as suggested by the high growth rates found in November. According to findings by Huwer *et al.* (2011), available samples from Multinet hauls revealed only few observations of larvae sampled above the thermocline assuming that larvae rather stay in intermediate, colder water layers with lower – but maybe higher quality - prey densities than dwelling in upper layers where there is more food but also a higher risk for thermal stress.

Higher growth rates in the earlier season of 2011 could not be explained by higher temperatures, as until June, temperatures were slightly higher in 2006 than in 2011. The differences in growth rates between the years indicate an overall high importance of high nutritional condition of Baltic cod larvae in order to increase growth, while lower temperatures might be beneficial in a food limited environment. Under increased temperatures, larvae need to encounter sufficient food in order to satisfy higher metabolic costs (Brett *et al.* 1979). Investigations by Brett *et al.* (1979) showed that restricted food intake has a marked influence on the scope of growth (G_{scope}) at any given temperature, hence effectively reducing the optimum temperature (T_{opt}) in food-limited environments. Olsen *et al.* (2016) found food-limited recruitment of North Sea cod to be strengthened in years of colder temperatures and that food availability in essence determines

which stock-recruitment model applies for the North Sea cod stock. Hence, the apparent shift from a non-limited to a food limited environment in the Bornholm basin (Hinrichsen *et al.* 2002) in combination with potentially increasing temperatures due to climate change might even decrease survival chances of Baltic cod larvae.

5.2.3 Growth performance

Given the fact that growth rates of larvae are temperature and size dependent, the more objective measure of the so-called growth performance (G_{pf}) was analysed. The growth performance gives information on the fraction of maximum growth realized by a larva at given size (dry weight) and temperature. The first attempt to calculate growth performance of field-caught Baltic cod larvae by Huwer *et al.* (2011) demonstrated the suitability of using literature derived growth rates as a reference in order to gain an objective measure of larval growth. However, it was suggested that further investigation and validation is needed. Since Baltic cod growth was compared with maximum growth rates from the literature, mainly derived by apparently faster growing populations of Atlantic cod, the growth performance of Baltic cod larvae within the Baltic Sea might be underestimated. Laboratory investigations on maximum growth rates of Baltic cod larvae fed in excess, but so far not available, might increase the validity of G_{pf} in terms of giving information on growth rates, subsequent survival changes and resulting potential recruitment success.

The inter- and intra-annual comparison of G_{pf} of Baltic cod larvae in 2006, 2007 (August) and 2011, demonstrated overall highest G_{pf} in 2011. However, Baltic cod larvae generally grew poorly compared to reference growth rates (G_{ref}). Folkvord (2005) proposed the hypothesis that surviving larvae in the field would grow close to their temperature- and size-dependent maximum growth rates. Former investigations by Huwer *et al.* (2011) already revealed Eastern Baltic cod larvae to grow poorly when comparing R/D-derived growth rates of Baltic cod larvae with G_{ref} from the literature. The small fraction of realized size- and temperature-independent maximum growth by Baltic cod larvae mitigates the influence of temperature and stresses the importance of potential food availability for first feeding larvae in the Bornholm Basin.

Highest percentage of larvae growing above a G_{pf} of 0.5 was found in May 2011, followed by larvae in August 2011, mainly presenting a growth performance between 0 and 0.5. With

increasing larval dry weight (DW) the spread in G_{pf} was reduced remarkably. While larvae < 125 µg DW in August 2011 showed strongest variability in G_{pf} primarily ranging from -0.68 to 1.86, larvae above this dry weight threshold demonstrated smaller differences and mainly positive growth performances. The number of larvae with a dry weight above 125 µg was 25% lower compared to the number of larvae < 125 µg. This might be due to high variability in the growth performance of small, first-feeding larvae competing for prey, indicating a critical period (Hjort 1914, 1926), where only larvae with higher G_{pf} survive and contribute to the potential recruitment.

5.3 Relation between nutritional condition and suitable prey fields

In order to find an explanation for the observed intra- and inter-annual variability in nutritional condition of Baltic cod larvae, having a potentially positive influence on larval growth, the seasonal occurrence of suitable prey fields in the years 2006 and 2011 was analysed. Voss *et al.* (2003) found larval cod to actively select for copepod nauplii independent of depth, with high preferences for *Pseudocalanus* spp. (gut contents of cod larvae consisted up to 91% of *Pseudocalanus* spp. nauplii). In this study, correlation matrices demonstrated best fits for the relationship between standardized R/D ratio of cod larvae and the cumulative occurrence of nauplii and copepodite stage 1 (C1). Hence, the analyses focused on relating the seasonal occurrence of nauplli and C1 stages of zooplankton species with the mere information on larval sRD. The inclusion of the rotifer genera into the correlation analyses was due to finding a possible explanation for unexpected good nutritional condition of cod larvae in November. Rotifer specimens have been found to be an alternative feed for first feeding larvae in aquaculture (O´Brien-McDonald *et al.* 2006, Park *et al.* 2006) and larval cod has been found to be opportunistic in order to cope with a lack of ideal food (Munk 1995, Rowlands *et al.* 2008, Seljeset *et al.* 2010). Unfortunately, data on zooplankton abundance and biomass in 2006 were only available for the months April, June and July. However, monthly means of larval nutritional condition reflected by standardized RNA/DNA ratios presented high correlations when relating to available mean abundance and biomass of early developmental stages of copepods and partly in addition of rotifers. The most explained variances for the relationship between nutritional condition of larvae and potential prey items was found for nauplii and C1 of the species

Pseudocalanus spp., while in some cases the explanatory power was slightly increased by the inclusion of other copepod species and the rotifer genera. Correlations between sRD ratios of cod larvae and prey fields were high, but as no larval stomach analyses were conducted in this present study, no causal relationship could be proven. Hence the $H_0$3 hypothesis (*The nutritional condition and growth of Baltic cod larvae has no relation to the seasonal occurrence, abundance and stage composition of dominant zooplankton species.*) could not be directly rejected but was further discussed based on pre-existing investigations on feeding preferences of Baltic cod larvae and distribution patterns of main zooplankton species found in the literature in order to allow for explanatory approaches on the importance of processes between feeding cod larvae and suitable prey fields.

The positive relation between nutritional condition of cod larvae and the abundance/biomass of the species *Pseudocalanus* spp. found in this study, is in accordance with several studies conducted on the preferred spectrum of prey organisms of Baltic cod larvae, demonstrating that nauplii and copepodite stages of the species *Pseudocalanus* spp. have a great importance as main food item and hence for the survival of cod larvae (Köster *et al.* 2003b, Voss *et al.* 2003, Hinrichsen *et al.* 2002, 2003b and 2005 and Schmidt 2006). The vertical distribution of early developmental stages of *Pseudocalanus* spp. (N + CI-CIII) has been found to be in shallower waters above the halocline mainly ranging between 20-50 m (Renz and Hirche 2006, Schulz *et al*. 2012). The vertical distribution of prey items is in accordance with other studies reporting feeding cod larvae mainly to be found between 20 and 45 m and positive larval growth above 45 m (Voss *et al.* 2003, Grønkjær *et al.* 1997, Huwer *et al.* 2011). Voss *et al.* (2003) also reported a significant incorporation of copepodite stages CI-CIII in the diets of larvae with a standard length of 6 to 7 mm. Even though correlations of the present data set decreased when including stages CII and CIII, there is a high potential that larvae > 6 mm (e.g. during August 2011) fed on these copepodite stages. Based on cod larvae sRD, highest correlations were found in combination with nauplii and C1 stages. Strong relations might indicate the high importance of both a temporal and spatial match between larvae and these stages. Between the years, the abundance of copepods showed no strong differences, with *Pseudocalanus* spp. being even less abundant in 2011. The main difference between the years was found in terms of the temporal occurrence of nauplii and following copepodite stages. While the peak in nauplii abundance in

2006 was found in April, no high larval abundance was found during sampling. However in 2011, both a nauplii peak and a moderate larval abundance were observed during the month May probably enabling cod larvae to fed on sufficient and suitable prey organisms resulting in a good nutritional condition reflected by high R/D values.

Contrary to expectations, the rotifer genera showed potential to increase the correlation between larval nutritional condition and potential food availability, mainly driven by high abundances and good larval nutritional condition in November 2011. Rotifers found within the Bornholm Basin mainly belong to the species *Synchaeta* spp. and range in size from 200 to 400 µm, while male organisms usually are smaller (> 90 µm; Jörg Dutz, pers. comm.). This size range corresponds to late nauplii/early copepodite stages, hence fitting into the spectrum of food items suitable for cod larvae. Within the Bornholm Basin, *Synchaeta* spp. has been found to build up a main peak in abundance in May (up to 26.000 individuals·m^{-3}, 20 to 80 % of all individuals in a net haul) often followed by a second increase in November, while abundances can vary strongly (Schulz *et al.* 2012, Jörg Dutz, pers. comm.). Further, specimens showed temperature tolerances up to 12°C and were usually found below the thermocline. Sampling during the night revealed main abundances between 20 and 60 m. The vertical distribution of rotifers and the preference for colder waters supports the possibility of an encounter between cod larvae and rotifers. Surprisingly, also the smallest size group of larvae (< 4.5 mm), which is assumed to be pre-feeding, demonstrated a good relation between the occurrence of early developmental stages of copepods (mainly *Pseudocalanus* spp.) and the rotifer genera. Grønkjær and Wieland (1997) excluded larvae under 4.5 mm from comparisons of nutritional condition and prey field in order to restrict the analysis to larvae which have a high probability of having initiated feeding on prey. The findings of this study might indicate an early transition of small Baltic cod larvae from endo- to exogenous feeding. For this size class also the relation between larval nutritional condition and abundance/biomass of rotifers was the highest found for the different size classes (R^2= 0.46; p= 0.09).

The improvement of nutritional condition of cod larvae was positively related to suitable prey fields. This might indicate that food limitation is one of the main factors influencing larval

growth and subsequent survival within the present main spawning ground for Eastern Baltic cod, the Bornholm Basin.

5.4 Relevancy for recruitment

Recruitment processes are highly complex and potential critical periods during the early life history acting as a bottleneck for recruitment can vary between the years and within the seasons depending on prevailing environmental conditions (Köster *et al.* 2001, Voss *et al.* 2001). In case of the Eastern Baltic cod stock, these critical periods may even be intensified as nutritional condition and growth rates are low compared to other Atlantic cod stocks, probably resulting in prolonged larval stage duration (Houde 1987, Otterlei *et al.* 1999, Huwer *et al*. 2011). Information on the seasonal and inter-annual variability in nutritional condition and growth performance of Eastern Baltic cod larvae may allow for a better understanding of recruitment processes and the formation of a year-class than the mere information on larval abundance. The dependency of first feeding and also established feeding Baltic cod larvae on finding suitable prey at both temporal and spatial scale directs the focus on the match/mismatch hypothesis (Cushing 1974, 1975, 1990) and seeks for further investigations bringing together both larval performance and the phenology of important prey species within the Bornholm Basin.

Even though a fraction of larvae might survive throughout an extended spawning season by feeding on alternative prey resources, temporary peaks in high quality food might indicate an optimal window for survival of Eastern Baltic cod larvae as it has been demonstrated for cod from Georges Bank (Buckley *et al.* 2010). Investigations by Buckley *et al.* (2010) suggested an optimal window for survival of early spawned cohorts of two Atlantic groundfish species (*Gadus morhua* and *Melanogrammus aeglefinus*) when seasonal temperature, predation pressure and even potential prey are at seasonal low levels. Later cohorts usually presented higher growth rates due to increased temperatures, but were often rapidly lost to higher predation pressure late in the season. As already mentioned before, in case of the Bornholm Basin, predation on cod larvae is assumed to be low and cohorts might rather be lost due to higher metabolic costs at increased temperature in combination with limited food later in the season. Processes like retention early in the season and dispersion late in the season into shallower waters with higher

food abundance (Hinrichsen *et al.* 2001, 2003b) might also enable (or inhibit) more than one optimal window for larval survival depending on larval drift (Huwer *et al.* 2014).

In order to determine processes influencing the survival during the early life history of Baltic cod it is not sufficient to only focus on the relation between larval nutritional condition and prey availability. Ottersen *et al.* (2014) indicated that there is no single life stage during which recruitment is determined. The mechanisms affecting a year-class strength and hence future recruitment are highly complex, complicating the disentanglement of individual factors and their relevance for successful recruitment. Especially within the semi-enclosed Baltic Sea, anthropogenic impacts, the dependency on climatic processes and the combined influence on the biotic and abiotic environment are reflected in high inter- and intra-annual fluctuations or even ecosystem regime shifts (e.g. Köster *et al.* 2003b, Alheit *et al.* 2005 Österbløm *et al.* 2007, Möllmann *et al.* 2009). The presented data and analyses only display a small piece of a jigsaw, scratching on the surface of understanding how biotic and abiotic conditions shape the number of recruits within a year. In this context, information on environmental conditions potentially influencing larval abundance and survival was collected (Table 9, Fig. 22, Fig. 23).

Assessment based time series on the standing stock of Eastern Baltic cod presented a positive relation between spawning stock biomass (SSB) and the number of recruits (at age 2) from 2005 onwards (Fig. 22b and c). The difference in SSB between 2006 and 2011 might be one of the reasons for higher larval abundances in 2011 and the high recruitment of the 2011 year-class at age 2 in 2013 (173 million). However, the number of recruits in 2013 was the highest since the early 1980s and adjacent years with similar or even higher SSB were not followed by comparable numbers of recruits. Besides, egg production in 2011 was relatively low compared to preceding years (Köster *et al.* unpubl.), providing further evidence that not the higher SSB but processes during the egg and/or larval stage resulted in the high recruitment success of that year-class. The stock-recruitment relationship in marine fish is highly variable (Hilborn and Walters 1992, Koslow 1992) and predictions on recruitment still count as a challenge (Houde 2008).

While information on the reproductive volume for Eastern Baltic cod by the ICES (2014) were summed over the three deep Basins of the Baltic Sea indicating a slightly higher RV in 2006 (not shown here), model run results by the Kiel Baltic Sea Ice Ocean Model (BSIOM, Lehmann and

Hinrichsen 2000) only for the Bornholm Basin demonstrated contrary results (Fig. 23a and d, Table 9). The BSIOM model enables a resolution of the RV giving additional information on e.g. oxygen dependent egg survival probability and larval migration activity depending on the age structure and hence the egg quality of a stock (Burkhard von Dewitz, pers. comm.).

Table 9: Summary of information for Eastern Baltic cod on I) the stock sizes and number of recruits at age 2, II) the Reproductive Volume (RV), III) the mean hydrological conditions within the RV and IV) the probability of egg survival and larval migration for the years 2006 and 2011 for both, cod at age 3 and 7 respectively. Data were either extracted from ICES information (ICES 2014) or derived from Baltic Sea Ice Ocean Model run results (BSIOM, Lehmann and Hinrichsen 2000). Legend: TSB (total stock biomass); SSB (spawning stock biomass); SLT (spawning layer thickness); RV (reproductive volume), ESP (oxygen dependent egg survival probability), LVMA (larval migration activity), BITS (Baltic International Trawl Survey) and CPUE (catch per unit effort). The BSIOM data were kindly provided by B. v. Dewitz.

	2006	**2011**	reference	calculation
TSB (tons)	121 176	150 693	ICES	BITS means of CPUE (no./h)
SSB (tons)	67 711	98 125	ICES	BITS means of CPUE (no./h)
Recruits (thousands)	130 353	173 859	ICES	BITS means of CPUE (no./h)
SLT (m)	19	17	ICES	mean of mean from May/August
temperature (°C)	5,4	4,79	ICES	mean of mean from May/August
oxygen (ml/l)	4,8	3,5	ICES	mean of mean from May/August
RV (km^3)	198	228	BSIOM	mean of monthly means
RV (age 3/7, km^2)	4439/8396	4791/8275	BSIOM	mean of monthly means
ESP(age 3/7, %)	9/70	86/90	BSIOM	mean of monthly means
LVMA (age3/7, %)	23/66	71/72	BSIOM	mean of monthly means

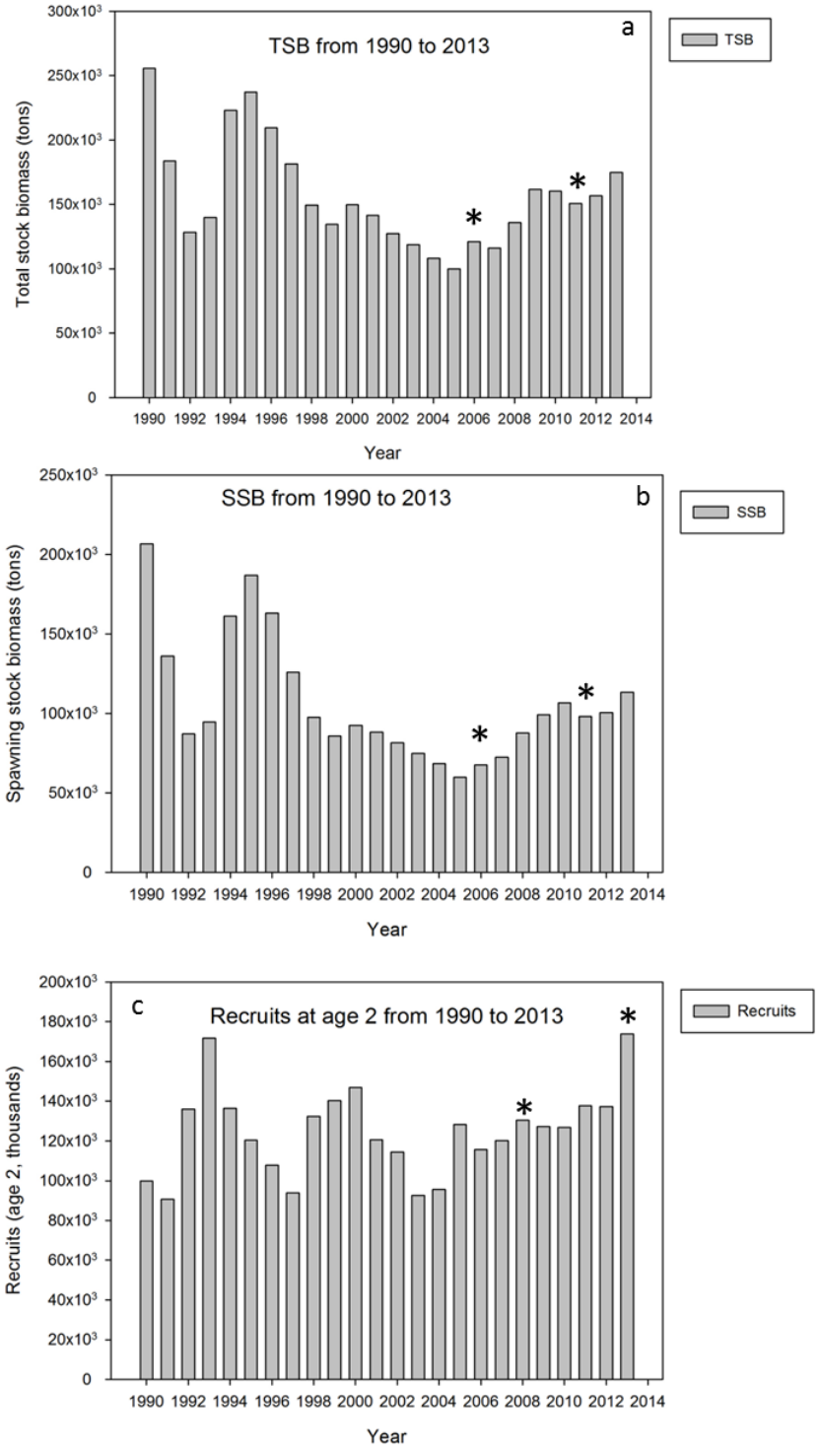

Fig. 22: Information on a) total stock biomass, b) spawning stock biomass and c) recruits (at age 2) from the year 1990 to 2013 with years of interest marked with "*" (2006,2011). Data were extracted from the ICES WGBFAS report (2014).

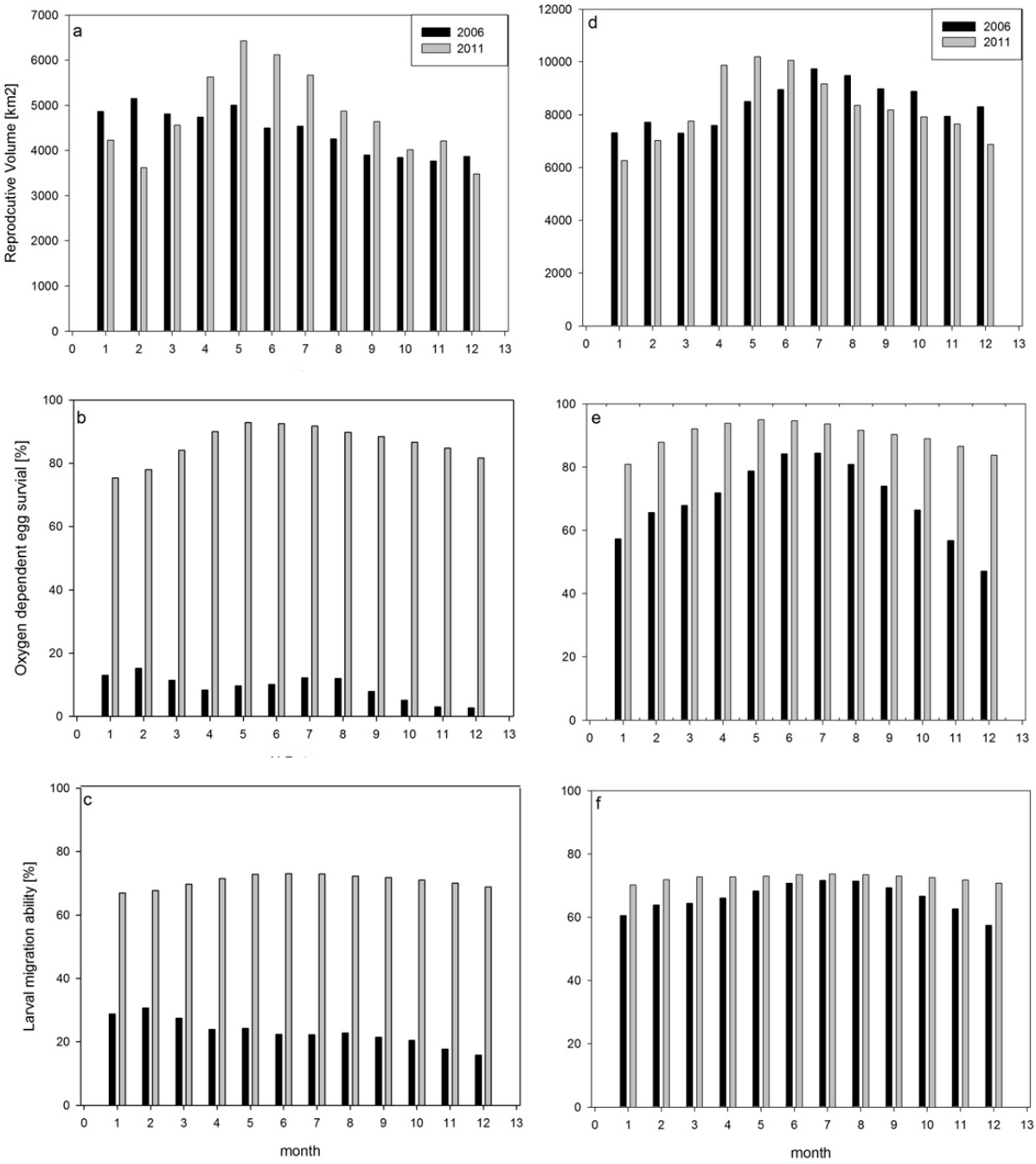

Fig. 23: Baltic Sea Ice Ocean Model (BSIOM, Lehmann and Hinrichsen 2000) run results estimating the a) and d) reproductive volume, b) and e) oxygen dependent survival probability of eggs and c) and f) larval migration ability for cod at age 3 and 7 respectively in the years 2006 and 2011. The data was kindly provided by Burkhard von Dewitz.

Interestingly, results of the BSIOM model runs for cod at age 7 showed only smaller differences between 2006 and 2011, but for younger cod at age 3, results presented much higher survival probabilities and migration activities for eggs and larvae in 2011 (Fig. 23 b,c at age 3 and e,f at age 7), mainly driven by a minor water inflow from the North Sea early in the year. The differences in hydrographical conditions for cod eggs spawned by distinct age-groups of Baltic cod seemed to be reduced from an age of 5 years (Burkhard von Dewitz, pers. comm.). The estimated oxygen dependent probabilities for egg survival and larval migration activity by the BSIOM model are strongly supported by the high differences in larval abundance between the years 2006 and 2011.

In 2014, a major water inflow from the North Sea into the Baltic Sea was described (Mohrholz *et al.* 2015). Even though this has improved conditions for egg survival and larval migration activity during recent years due to increased oxygen content within the RV, no high abundances of cod larvae in the Bornholm Basin have been found in the post-inflow years 2014 and 2015 (Köster *et al.* unpubl., Bastian Huwern and Burkhard von Dewitz, pers. comm.). Köster *et al.* (2005) described a similar situation for the mid-1990s, where recruitment was low despite of improved hydrographic conditions for egg survival, mainly driven by food-limitation. Further, Eero *et al.* (2015) summarized the knowledge of recently unusual changes in cod biology and ecosystem conditions, indicating the Baltic cod stock to be in distress. Unfavourable developments stated in the study included e.g. the low nutritional condition and absence of larger cod (< 38cm). Hence, a combination of the loss of older and bigger cod in the system, a reduced mean weight at age, a restricted area and quality of the reproductive volume due to stagnation periods, and food-limitation of larvae might even increase mortality rates during the early life-history of Baltic cod in future years.

Analyses of this study demonstrated, that processes during the larval phase can vary substantially depending on biotic and abiotic conditions, hence potentially causing high variability in recruitment of Eastern Baltic cod. Provided information on stock size and stock-structure dependent egg survival probability additionally revealed the complexity of cascading processes acting on recruitment. Therefore, further investigations need to be conducted in order to disentangle single and combined effects.

In the present study, only mean values over the years and months were compared, but observation of data revealed strong spatial differences between stations, especially in terms of prey field abundances and temperature values. Future investigations should focus on the nutritional condition and growth of cod larvae in relation to food availability preferably both, horizontally and vertically resolved. Information could be related to the influence of environmental conditions at a given station and the time of sampling. Analyses should be combined with and validated by bio-physical and individual-based modeling (IBM). Hinrichsen *et al.* (2003b) already demonstrated the low survival probability of cod larvae when the copepod species *Pseudocalanus* spp. was low in abundance or even removed from the system. Increased knowledge on the coherences between physical processes, the number and distribution of prey items and consequently the behaviour (Jørgensen *et al.* 2014) and nutritional condition of Baltic cod larvae might improve the predictions of present models.

Huwer *et al.* (2011) addressed the problem of generally low abundance of cod larvae in samples for biochemical analyses. High abundances of Baltic cod larvae in 2011 were uncommon and similarly high numbers of larvae for comparative analyses are usually difficult to achieve. However, time series should be analysed even though numbers of larvae might be low. Köster *et al.* (2001) demonstrated the sufficient use of relatively simple models to predict recruitment of 0-group cod in the Baltic Sea. Increased knowledge about variability in growth and survival may provide important input to refine biophysical IBMs and environmentally sensitive stock-recruitment models and could potentially improve estimations of recruitment and future spawning stock biomass. This may be of particular importance during a time, where analytical assessment of the stock is in reconditioning and the stock structure seems to be uncertain (Eero *et al.* 2015).

6 Conclusion

In conclusion, this study demonstrated that cod larvae originating from the Bornholm Basin varied in abundance, nutritional condition and growth performance within and between years. Nutritional condition and growth increased with increasing larval size. Overall, Baltic cod larvae grew poorly compared to reference growth rates from the literature. Cod larvae in 2011 displayed higher nutritional condition and growth performance than compared to 2006 and 2007. Contrary to expectations, larval nutritional condition and growth rates were relatively high in November during both seasons 2006 and 2011. Analyses of the nutritional condition of Baltic cod larvae in relation to the main mesozooplankton occurrence and composition in 2006 and 2011 indicated strong correlations between larval nutritional condition and early developmental stages of the main copepod species, with highest importance of *Pseudocalanus* spp.. Specimens of the rotifer genera showed potential to increase the explanatory power when relating larval nutritional condition and prey field, mainly driven by high abundances of rotifers and relatively good nutritional condition of cod larvae during November 2011.

This study provides support from the field that a spatial and temporal match of the emergence of feeding cod larvae with the availability of early developmental stages of the species *Pseudocalanus* spp. as suitable prey apparently narrows the window for relatively good larval nutritional condition and growth performance and hence potentially influences larval survival and year-class strength of Eastern Baltic cod.

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9 Declaration of Authorship

Herewith I certify that the present thesis, apart from the consultation of my supervisors, was independently prepared by me. No other than the indicated resources and references were used. This thesis was presented to no other place within the scope of an examination procedure. The written thesis is identical with the electronic one.

Kiel, 1st of July

Luisa Berghoff